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<p>(21) International Application Number: PCT/AU94/00297 (22) International Filing Date: 3 June 1994 (03.06.94) (30) Priority Data: PL 9181 3 June 1993 (03.06.93) AU (71) Applicant (for all designated States except US): THE UNIVERSITY OF SYDNEY [AU/AU]; Parramatta Road, Sydney, NSW 2006 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): ROUFOGALIS, Basil, Don [AU/AU]; 40 Pymble Avenue, Pymble, NSW 2031 (AU). DUKE, Colin, Charles [AU/AU]; 19 Titania Street, Randwick, NSW 2031 (AU). LI, Qian [CN/AU]; 1/3 Carrington Street, Lewisham, NSW 2049 (AU). (74) Agent: GRIFFITH HACK & CO.; P.O. Box 4164, Sydney, NSW 2001 (AU).</p>		<p>(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.</p>
<p>(54) Title: USE OF NATURAL PRODUCTS AND RELATED SYNTHETIC COMPOUNDS FOR THE TREATMENT OF CARDIO-VASCULAR DISEASE (57) Abstract Use of a compound of formula (I), wherein Ar is an aromatic ring system comprising one or more optionally substituted phenyl rings optionally linked to or fused with one or more other optionally substituted phenyl rings or one or more 5 or 6-membered, optionally substituted heterocyclic rings wherein the heteroatom is oxygen; and wherein the ring system comprises 1-4 phenyl rings and wherein Ar can be linked to another Ar via a group X wherein the Ar is independently selected; where X is optionally substituted C₁₋₂₀alkylene, C₂₋₂₀alkenylene or C₂₋₂₀alkynylene; R is hydrogen; C₁₋₂₀alkenyl, C₂₋₂₀alkynyl, C₂₋₂₀alkanoyl, C₂₋₂₀alkynoyl, each of which can be optionally substituted; R₁ is independently selected and is hydrogen; optionally substituted C₁₋₁₂alkyl, C₂₋₁₂alkenyl, C₂₋₁₂alkynyl; -COOR' -NR'R', halogen, -OR', -COR', -CONR'R', =O, -SR', -SO₃R', -SO₂NR'R', -SOR', -SO₂R', -NO₂, -CN, glycoside, silyl; where R' is independently hydrogen; alkyl, alkenyl or alkynyl each optionally substituted; and where two groups R₁ can be joined; wherein the optional substituents are one or more independently selected from C₁₋₁₀alkyl, C₂₋₁₀alkenyl, C₂₋₁₀alkynyl; -COOR'' -NR''R'', halogen, OR'', -COR'', -CONR''R'', -SR'', =O, -SO₃R'', -SO₂NR''R'', -SOR'', -SO₂'', -NO₂, -CN; wherein R'' is independently hydrogen, alkyl, alkenyl, or alkynyl; n = 1, 2 or 3; m = 1, 2, 3 or 4; or a pharmaceutically acceptable derivative thereof in the manufacture of a medicament for inhibiting the action of Ca²⁺-ATPase enzymes. Certain compounds of formula (I) and 8-lavandulyl flavone pharmaceutical formulations are new.</p> <div style="text-align: center;">  <p>(I)</p> </div>		

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USE OF NATURAL PRODUCTS AND RELATED SYNTHETIC COMPOUNDS
FOR THE TREATMENT OF CARDIOVASCULAR DISEASE

Technical Field

5 The present invention relates to the use of naturally occurring phenolic compounds and related synthetic compounds in the treatment or prophylaxis of cardiovascular disease and to novel phenolic compounds and the use thereof in the treatment or prophylaxis of cardiovascular disease.

10 Background Art

Cardiovascular disease is a serious health problem and a major cause of death in Australia and most developed countries. It has been reported that calcium is central to cardiovascular function, in that the
15 calcium ion controls the contraction of heart muscle and the tone of blood vessels. Certain drugs have been used to increase intracellular calcium in order to stimulate the failing heart (cardiotonic agents). The major drugs used for congestive heart failure in the past are derived
20 from digitalis, found naturally in plants such as foxglove. Their action to raise intracellular calcium, however, is indirect, as they inhibit Na^+, K^+ -ATPase which results in an increase in intracellular Na^+ , which then in turn stimulates the inflow of extracellular calcium
25 and in turn stimulates the failing heart. These drugs are not ideal as they are toxic at doses only slightly higher than therapeutic cardiotonic concentrations. There has been an active search for alternative cardiotonic agents in recent years and there is still a
30 need for effective drugs to treat and prevent various aspects of cardiovascular disease.

Ca^{2+} has a variety of functions in most animal cells. The concentration of free calcium ion (Ca^{2+}) in the cytoplasmic space acts as an intracellular messenger in
35 both electrical and non-electrical excitable cells. The important role of Ca^{2+} is in relation to cellular contraction, and proliferation especially contraction and relaxation of the heart.

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The movement of Ca^{2+} across cells is regulated by number of mechanisms. If there are means that can pharmacologically manipulate these processes then the level of free intracellular Ca^{2+} may be altered, resulting in a change in cellular response.

There are number of calcium pools which contribute to the concentration of Ca^{2+} in the cytoplasmic space. Two major important pools are namely, the extracellular pool and the internal store, the so-called sarcoplasmic reticulum (SR) store.

The entry of extracellular Ca^{2+} , down its electrochemical gradient, not only raises the level of intracellular Ca^{2+} but also initiates the release of Ca^{2+} from the SR store. This phenomenon explains the rapid contraction of cells. The rise of intracellular Ca^{2+} is compensated by a number of mechanisms to remove Ca^{2+} from the cytoplasmic space, either by extruding the Ca^{2+} out of the cell through the Ca^{2+} pump, which is biochemically coupled to Ca^{2+} -ATPase, and the $\text{Ca}^{2+}/\text{Na}^{+}$ exchanger, and by sequestering of Ca^{2+} back into the SR store through SR Ca^{2+} -ATPase. These removal mechanisms are energy-dependent processes that utilise ATP as the energy source.

The present inventors have found that a range of naturally occurring phenols and related synthetic compounds manipulate the plasma membrane Ca^{2+} -ATPase process named, hereafter, as the plasma membrane Ca^{2+} -ATPase. It is anticipated that they may also alter the SR Ca^{2+} -ATPase, given the similarity of this enzyme to this plasma membrane Ca^{2+} -ATPase. Compounds discovered can inhibit the plasma membrane Ca^{2+} -ATPase causing an increased level of free Ca^{2+} inside cell. At the same time, some of the compounds may be chosen to stimulate the SR Ca^{2+} -ATPase, thereby increasing Ca^{2+} uptake into the internal SR store and making more Ca^{2+} available for release from the SR. The overall effect of these compounds is to increase the rate of contraction as well as the force of contraction of the heart cells, and particular of the failing heart.

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It has been reported that a number of nonspecific reagents inhibit plasma membrane Ca^{2+} -ATPase. It has also been reported that a number of long chain alcohols, hemin and nonhemin iron and fatty acids partially inhibit Ca^{2+} -ATPase of erythrocyte membrane. The retinoids have been shown to have anti-calmodulin effects and therefore indirect effects on the Ca^{2+} -pump enzyme. The sesquiterpene lactone thapsigargin was found to be a specific inhibitor of Ca^{2+} -ATPase of skeletal muscle endoplasmic reticulum.

Disclosure of the Invention

In one aspect, the present invention provides the use of a compound of formula (I), a pharmaceutically acceptable salt or ester thereof in the inhibition of Ca^{2+} -ATPase enzymes



wherein Ar is an aromatic ring system comprising one or more optionally substituted phenyl rings optionally linked to or fused with one or more other optionally substituted phenyl rings or one or more 5 or 6-membered, optionally substituted heterocyclic rings wherein the heteroatom is oxygen; and

wherein the ring system comprises 1-4 phenyl rings and

wherein Ar can be linked to another Ar via a group X wherein the Ar is independently selected;

where X is optionally substituted C_{1-20} alkylene, C_{2-20} alkenylene or C_{2-20} alkynylene;

R is hydrogen; C_{1-20} alkyl, C_{2-20} alkenyl, C_{2-20} alkynyl, C_{2-20} alkanoyl, C_{2-20} alkenoyl, C_{2-20} alkynoyl, each of which can be optionally substituted;

R_1 is independently selected and is hydrogen; optionally substituted C_{1-12} alkyl, C_{2-12} alkenyl, C_{2-12} alkynyl; $-\text{COOR}'$, $-\text{NR}'\text{R}'$, halogen, $-\text{OR}'$, $-\text{COR}'$, $-\text{CONR}'\text{R}'$,

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=O, -SR', -SO₃R', -SO₂NR'R', -SOR', SO₂R', -NO₂, -CN, glycoside, silyl;

where R' is independently hydrogen; alkyl, alkenyl or alkynyl each optionally substituted; and

5 where two groups R₁ can be joined;

wherein the optional substituents are one or more independently selected from C₁₋₁₀alkyl, C₂₋₁₀alkenyl, C₂₋₁₀alkynyl; -COOR", -NR"R", halogen, -OR", -COR", -CONR"R", -SR", =O, -SO₃R", -SO₂NR"R", -SOR", -SO₂R", -NO₂, -CN; 10 wherein R" is independently hydrogen, alkyl, alkenyl, or alkynyl;

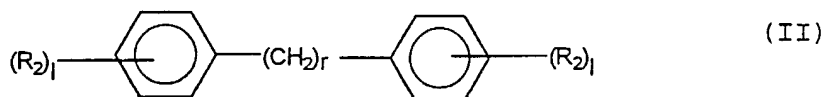
n = 1, 2 or 3

m = 1, 2, 3 or 4.

15 Preferably, the use of compounds of formula (I) is in the inhibition of plasma membrane Ca²⁺-ATPase.

In a second aspect, the present invention provides the use of a compound of formula (I), a pharmaceutically acceptable salt or ester thereof in the treatment or prophylaxis of cardiovascular disease.

20 In a third aspect, the present invention provides novel compounds of formulae (II), (III), (IV), (V), (VI) or pharmaceutically acceptable derivatives thereof:



where

25 l = 1, 2 or 3

r = 7-14 when l = 1

8-16 when l = 2 or 3

R₂ is (1) 2-hydroxy

(2) 2-hydroxy-4'-hydroxy

30 (3) 2-hydroxy-3-methyl

(4) 4-hydroxy-3-methyl

(5) 2,4-dihydroxy

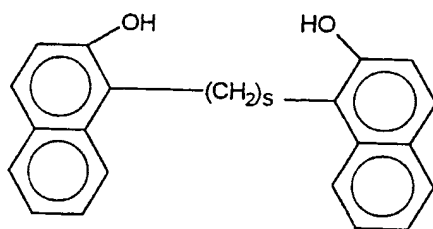
(6) 3,5-dihydroxy-4-methyl

- 5 -

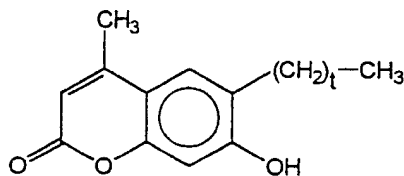
(7) 2,6-dihydroxy-4-methyl

(8) 2,4-dihydroxy-3-methyl

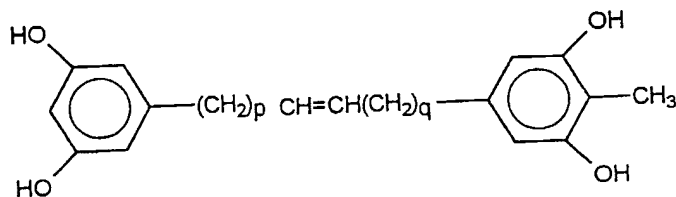
(9) 3-hydroxy-4-methyl

provided that (1) when R_2 is 2-hydroxy and $l = 1$ then r is not 7-10 and(2) when $l = 3$ and R_2 is 3,5-dihydroxy-4-methylthen n is not 14 and(3) when $l = 2$ and R_2 is 2-hydroxy-3-methylthen n is not 10(4) when $l = 2$ and R_2 is 2,4-dihydroxythen r is not 8-10 and 13 and(5) when $l = 2$ and R_2 is 4-hydroxy-3-methylthen r is not 10;

(III)

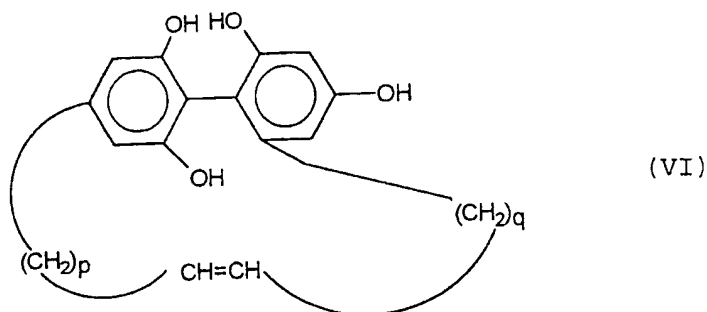
where $s = 8-16$ 

(IV)

where $t = 6-15$ 

(V)

where $p + q = 12$

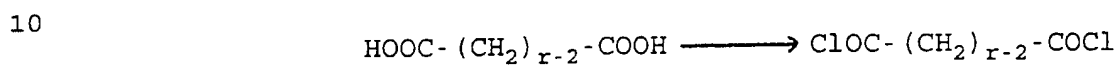


where $p + q = 12$

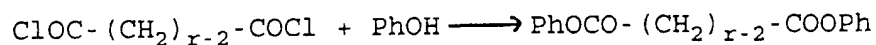
In a fourth aspect, the present invention provides a method of preparing compounds of formula (II) which comprises

(a) where $l = 1$ and R_2 is OH

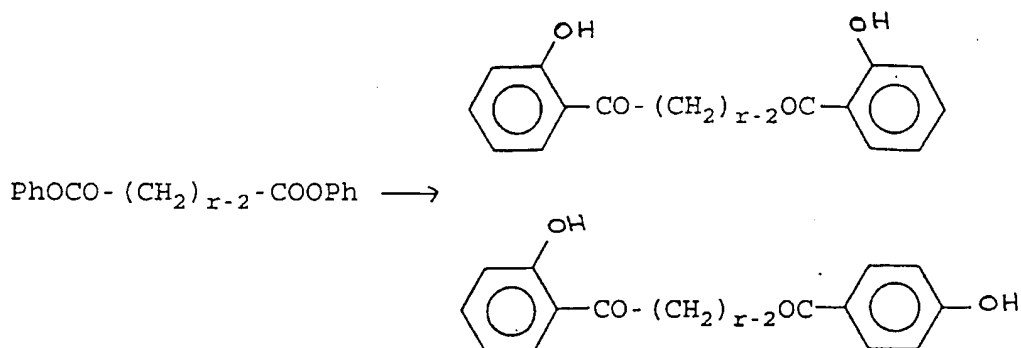
(i) treating the corresponding diacid with a suitable agent to provide the acid dichloride as follows



(ii) treating the corresponding acid dichloride with phenol as follows

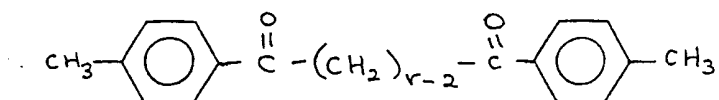


15 (iii) rearrangement of the diacyl groups as follows

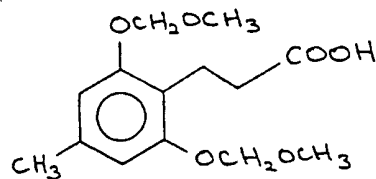


- 7 -

- (iv) followed by reduction of the acyl groups to provide compounds of formula (II);
- (b) where $l = 2$, R_2 is OH
- (i) treating the corresponding diacid with zinc chloride and resorcinol; and
- (ii) followed by reduction of the acyl groups to provide compounds of formula (II);
- (c) where $l = 2$, R_2 is 2-hydroxy-3-methyl carrying out steps (i)-(iv) in (a) above except in (ii) phenol is replaced with *o*-cresol;
- (d) where $l = 2$ and R_2 is 3-hydroxy-4-methyl
- (i) nitrating the corresponding diketo compound of formula



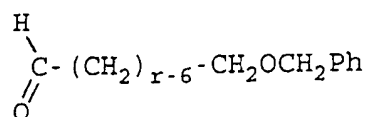
- to give the corresponding bis-3-nitro compound
- (ii) reducing the bis-3-nitro compound to give the bis-3-amino compound followed by
- (iii) diazotisation and hydrolysis to give the bis-3-hydroxy compound
- (iv) followed by reduction of the keto groups to give the desired bis-3-hydroxy compound;
- (e) where $l = 3$ and R_2 is 2,6-dihydroxy-4-methyl
- (i) treatment of a compound of formula



- with LDA to give the dianion followed by
- (ii) treatment with the desired protected

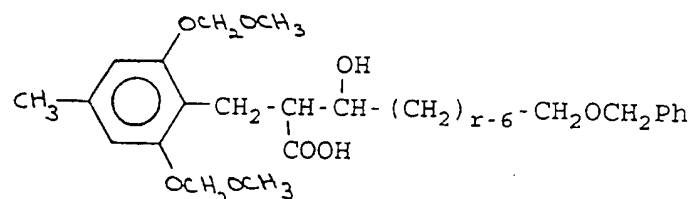
- 8 -

alkane aldehyde of formula



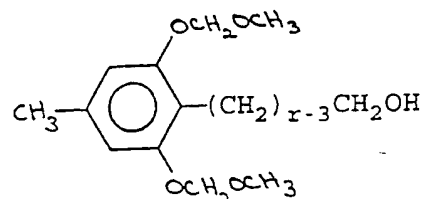
to give

5

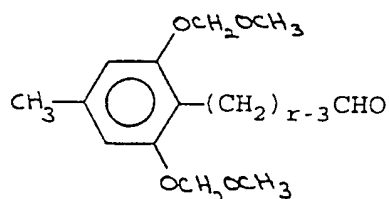


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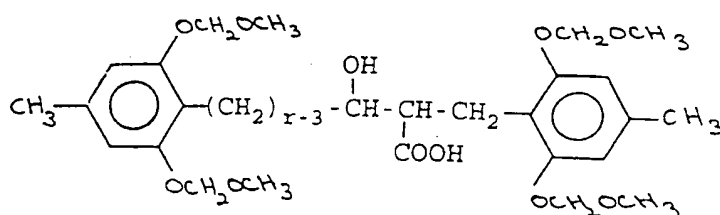
(iii) dehydrative decarboxylation followed by reduction to give an intermediate product of formula



(iv) oxidation to give



(v) followed by treatment with the dianion from step (i) to give



15

(vi) dehydrative decarboxylation followed by deprotection and reduction to give the

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desired product;

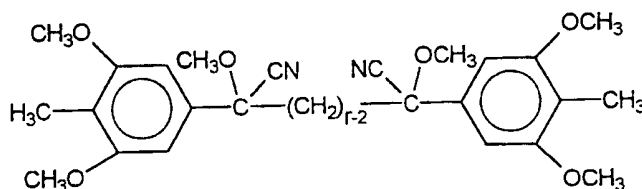
(f) where $l = 3$ and R_2 is 3,5-dihydroxy-4-methyl

5

(i) treatment of α -N,N-dimethylamino- α -cyano-(3,5-dimethoxy-4-methyl)benzylidene in tetrahydrofuran and hexamethylphosphoramide (HMPA) with lithium diisopropylamide (LDA) to give the anion followed by

10

(ii) treatment with α,ω -dibromoalkanes to give



(iii) refluxing with 30% aqueous oxalic acid to give the corresponding diacyl compound

(iv) reduction of the acyl groups

15

(v) followed by demethylation with hydrogen bromide in acetic acid to provide compounds of formula (II);

(g) where $l = 3$ and R_2 is 2,4-dihydroxy-3-methyl

20

(i) carrying out steps (i) and (ii) in (b) except in (i) resorcinol is replaced with 2-methylresorcinol;

(h) where $l = 2$ and R_2 is 4-hydroxy-3-methyl

25

(i) treating the corresponding diacid with ortho-cresol in the presence of polyphosphoric acid to give the corresponding diacyl compound

(ii) followed by reduction the acyl groups to provide compounds of formula (II).

In a fifth aspect, the present invention provides a method of preparing compounds of formula (III) which comprises

30

(i) treating the corresponding diacid with a

- 10 -

- suitable agent to provide the acid dichloride
- (ii) treating the corresponding acid dichloride with 2-naphthol followed by
 - (iii) rearrangement of the diacyl groups and
 - 5 (iv) followed by reduction of the acyl groups to provide compounds of formula (III)

In a sixth aspect, the present invention provides a method of preparing compounds of formula (IV) which comprises treatment of 4-alkylresorcinols with ethyl
10 acetoacetate in the presence of an acid catalyst to give compounds of formula (IV).

Preferably, Ar is phenyl, naphthalene, anthracene, naphthacene or phenanthrene. More preferably, Ar is phenyl.

15 All alkyl, alkenyl or alkynyl carbon chains can be straight or branched chain.

Halogen includes bromo, chloro, fluoro or iodo.

The 5 or 6-membered heterocyclic ring can be saturated, partially unsaturated or unsaturated.

20 Pharmaceutically acceptable derivatives include pharmaceutically acceptable ethers, esters and acid addition salts.

In the preparation of compounds of formula (II), preferably, the acid dichloride is formed by treating the
25 corresponding diacid with thionyl chloride. However, any other suitable agent can be used.

Preferably, in the preparation of compounds of formula (IV) the acid catalyst is boron trifluoride etherate or the like.

30 Preferably, the rearrangement of the acyl groups to the required positions on the phenyl ring is carried out using CS_2 and AlCl_3 as catalyst. The catalyst can generally be any Lewis acid such as BF_3 , ZnCl_2 , FeBr_3 or the like.

35 The reduction of the acyl group is preferably carried out using amalgamated zinc and a mixture of hydrochloric acid and optionally acetic acid.

Nitration is preferably carried out in the usual way

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using a combination of nitric acid and sulfuric acid ($\text{HNO}_3/\text{H}_2\text{SO}_4$). The reduction of the nitrate to the amine is preferably carried out using stannous chloride and hydrochloric acid (SnCl_2/HCl).

5 Diazotisation is preferably carried out by treatment with aq. $\text{H}_2\text{SO}_4/\text{NaNO}_2$ and hydrolysis is usually carried by using 10% H_2SO_4 .

Dehydrative decarboxylation is preferably carried out by using N-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinoline and deprotection by using p-TsOH/MeOH.

10 In another aspect, the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable derivative thereof in the manufacture of a medicament for inhibition of Ca^{2+} -ATPase enzymes, preferably, plasma membrane Ca^{2+} -ATPase.

In yet another aspect, the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable derivative thereof in the manufacture of a medicament for the treatment or

20 prophylaxis of cardiovascular disease.

Brief Description of the Drawings

Figure 1 is a graph showing concentration dependency of inhibition of erythrocyte plasma membrane of synthetic alkyl phenols.

25 □ 2-nonylphenol ▲ 3-nonylphenol
 ● 2-octylphenol ○ 4-octylphenol
 ▼ 2-decylphenol ■ 4-nonylphenol
 ▼ 4-decylphenol

30 Figure 2 is a graph showing concentration dependency of inhibition of erythrocyte plasma membrane of synthetic bis(hydroxyphenyl)alkanes.

● 1,10-bis(2-hydroxyphenyl)decane
□ 1,12-bis(2-hydroxyphenyl)dodecane
○ 1,14-bis(2-hydroxyphenyl)tetradecane
35 ▲ 1-(2-hydroxyphenyl)-10-(4-hydroxyphenyl)decane
 ▼ 1-(2-hydroxyphenyl)-12-(4-hydroxyphenyl)dodecane

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△ 1-(2-hydroxyphenyl)-14-(4-hydroxyphenyl)
tetradecane

■ 1,10-bis(4-hydroxyphenyl)decane

▽ 1,14-bis(4-hydroxyphenyl)tetradecane

5 Figure 3 is a graph showing concentration dependency
of inhibition of erythrocyte plasma membrane of
resorcinol derivatives.

□ ethyl 3,5-dibromo-2,4-dihydroxy-6-nonylbenzoate

● ethyl 3,5-dibromo-2,4-dihydroxy-6-decylbenzoate

10 △ ethyl 2,4-dihydroxy-6-nonylbenzoate

▲ ethyl 2,4-dihydroxy-6-decylbenzoate

Figure 4 is a graph showing concentration dependency
of inhibition of erythrocyte plasma membrane of tert-
butylphenols.

15 ● 2,3-di-tert-butyl-4-methoxyphenol

□ 2,6-di-tert-butylphenol

▲ 2,4,6-tri-tert-butylphenol

Figure 5 is a graph showing concentration dependence
of Ca^{2+} -ATPase inhibition of 2-nonylphenol derivatives.

20 ● 2-nonylphenol/ O (+CaM)

▽ 3-methyl-6-nonylphenol/ ▽ (+CaM)

■ 4-methyl-6-nonylphenol/ □ (+CaM)

● 4-nitro-2-nonylphenol/ O (+CaM)

▽ 4-bromo-2-nitro-6-nonylphenol (+CaM)

25 □ 2-bromo-4-nitro-6-nonylphenol (+CaM)

▲ 4-bromo-2-nonylphenol/ ▲ (+CaM)

■ 4-nonylresorcinol

Figure 6 is a graph showing concentration dependence
of Ca^{2+} -ATPase inhibition of α,ω -bis[2-hydroxy(3,4 and 5-
30 methyl)phenyl]alkanes.

● 1,8-bis(2-hydroxyphenyl)octane/ O (+CaM)

▽ 1,9-bis(2-hydroxyphenyl)nonane/ ▽ (+CaM)

■ 1,10-bis(2-hydroxyphenyl)decane/ □ (+CaM)

▲ 1,12-bis(2-hydroxyphenyl)dodecane/ ▲ (+CaM)

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- ◆ 1,10-bis(2-hydroxy-3-methylphenyl)decane/ ◇ (+CaM)
- 1,10-bis(2-hydroxy-4-methylphenyl)decane/ ○ (+CaM)
- 1,10-bis(2-hydroxy-5-methylphenyl)decane/ □ (+CaM)

Figure 7 is a graph showing concentration dependence
 5 of Ca^{2+} -ATPase inhibition of α,ω -bis[2-4-dihydroxyphenyl]alkanes.

- 1,8-bis(2,4-dihydroxyphenyl)octane/ ○ (+CaM)
- ▼ 1,10-bis(2,4-dihydroxyphenyl)decane/ ▼ (+CaM)
- 1,11-bis(2,4-dihydroxyphenyl)undecane/ □ (+CaM)
- 10 ▲ 1,12-bis(2,4-dihydroxyphenyl)dodecane/ ▲ (+CaM)

Figure 8 is a graph showing concentration dependence
 of Ca^{2+} -ATPase inhibition of α,ω -bis[hydroxy(methyl)phenyl
 and naphthyl]decanes.

- ◆ 1,10-bis(3-hydroxyphenyl)decane/ ◇ (+CaM)
- 15 ● 1,10-bis(3-hydroxy-4-methylphenyl)decane/ ○ (+CaM)
- ▼ 1,10-bis(4-hydroxy-3-methylphenyl)decane/ ▼ (+CaM)
- 1,10-bis(2-hydroxy-1-naphthyl)decane/ □ (+CaM)
- ▲ 1,1-bis(2-hydroxyphenyl)decane/ ▲ (+CaM)

Figure 9 is a graph showing concentration dependence
 20 of Ca^{2+} -ATPase inhibition of phenolic natural products.

- 5,7,2',6'-tetrahydroxy-8-lavandulylflavanone
- 5,7,6'-trihydroxy-8-lavandulylflavanone
- ▲ 5,2',6'-trihydroxy-8-lavandulyl-7-methoxyflavanone

Modes for Carrying Out the Invention

25 Compounds of formula (I) encompasses naturally occurring compounds as well as synthetically prepared related compounds. The naturally occurring compounds of formula (I) were extracted from endemic Australian plants of the Proteaceae family according to standard literature
 30 procedure, for example, see Ritchie E, Taylor W C and Vautin S T K (1965), Chemical studies of the Proteaceae I Aust.J.Chem., 18, 2015-2020; Rasmussen M, et al (1968) Chemical studies of the Proteaceae III. Aust.J.Chem., 21, 2989-3000 and Ridley D D, et al (1970) Chemical studies

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of the Proteaceae IV. Aust.J.Chem., 23, 147-183.

Long-chain alkyl phenols were extracted from *Grevillea* and *Persoonia*. Novel compounds having the structural formulae (II) and (III) were extracted and
5 isolated from *Grevillea robusta* collected in Sydney, Australia. A voucher specimen is available for inspection in the Department of Pharmacy, at the University of Sydney. Briefly, a sample of two kilograms was extracted by percolation with chloroform/ethanol
10 (1:1) for three days. After concentration of the extract in vacuo, the residue was chromatographed using silica gel short column vacuum chromatography.

Other compounds falling within the scope of formula (I) were prepared according to literature procedures or
15 are commercially available. Starting materials for the syntheses are commercially available or are prepared according to literature procedures.

The 2- and 4-substituted alkylphenols and α,ω -bis(hydroxyphenyl)alkanes were prepared by published
20 methods (see E Miller and W H Hartung, Organic syntheses (1943), collective volume II, 543-545 and R R Read and J Wood, Organic syntheses (1955), collective volume III, 444-446). The syntheses were performed in three consecutive stages: firstly, the formation of the ester
25 from an acid and phenol; secondly, the rearrangement of the acyl group, using $AlCl_3$ as catalyst, to the 2- and 4-positions relative to the hydroxy group on the phenol ring and thirdly, the reduction of the acyl group using amalgamated zinc and hydrochloric acid. The
30 rearrangement is a time dependent reaction and generally a short reaction period provides the acyl group at the 4-position relative to the hydroxy group.

Short column vacuum chromatography was used at each stage of synthetic reactions to separate and purify the
35 products from the reaction mixture. TLC methods were also employed to identify the products and to determine the eluent solvent required for column chromatography. The products at each stage were also characterised by NMR

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and CI-MS analysis.

The 3-substituted phenol derivatives were prepared according to another method, described by Itokawa H, et al (1989), A quantitative structure activity relationship for antitumor long-chain phenols from *Ginkgo biloba* L. Chem.Pharm.Bull. 37, 1619-1621. This method was used to prepare 3-nonylphenol with the aim of comparing its Ca^{2+} -ATPase inhibitory activity with those of the 2-and 4-substituted isomers. Hence, the importance of substitution on the phenol ring for Ca^{2+} -ATPase inhibition could be determined.

The bisphenol compounds were prepared, isolated and purified using procedures similar to those described above for alkylphenol, except that the preparation of esters was carried out in two separate steps.

For compounds of formula (II) where R_2 is 2-hydroxy-3-methyl the preparation is similar to that of the bishydroxyphenyl alkanes except instead of phenol, ortho-cresol is used following the procedure of K Kakemi et al in Antioxidants III Yakugaku Zasshi 86 [9], 791-796 (1966). 3-hydroxy-4-methyl compounds of formula (II) are prepared by a similar method to that used to prepare α,ω -bis(3-hydroxyphenyl)alkanes following the procedure of K. Kakemi et al. above except that α,ω -bis(4-methylphenyl)- α,ω -alkanediones are used instead of α,ω -bisphenyl- α,ω -alkanediones. 2,4-dihydroxy compounds of formula (II) are prepared by the reaction of resorcinol with the corresponding dicarboxylic acids in the presence of zinc chloride to give the intermediate alkanediones which are then reduced.

Compounds of formula (II) where R_2 is 2,6-dihydroxy-4-methyl are prepared by a method similar to that used to prepare grifolin following the procedure of S Ohta et al A total synthesis of grifolin Chem.Pharm.Bull. 36 [6] 2239-2243 (1988).

The starting compound in step (e)(i) is obtained according to the procedure of S Ohta et al referred to above. The aldehyde at step (e)(ii) is obtained by

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alkylating commercially available compounds of formulae
X-CH₂-(CH₂)_{r-6}CH₂-OR where R=H, X=Cl or Br with benzyl
bromide (or iodide) to give an intermediate product where
R=CH₂Ph followed by hydrolysis to give HOCH₂(CH₂)_{r-6}
5 CH₂OCH₂Ph followed by oxidation to give OHC(CH₂)_{r-6}
CH₂OCH₂Ph.

Compounds of formula (II) where R₂ is 3,5-dihydroxy-
4-methyl are prepared by a procedure similar to that
described by K. Takahashi et al., J. Org. Chem. 1983, 48,
10 1909-1912.

Compounds of formula (II) where R₂ is 2,4-dihydroxy-
3-methyl are prepared by a procedure similar to that
described by J. von Braun et al., Ber. (1941), 74B, 1772-
1783 except that 2-methylresorcinol is used instead of
15 resorcinol.

Compounds of formula (III) are prepared by a method
similar to that used to prepare α,ω-bis(2-
hydroxyphenyl)alkanes following the procedure of K.
Kakemi et al. Yakugaku Zasshi (1966), 86, 791-796.

20 Compounds of formula (IV) are prepared by a method
similar to that used to prepare 6-alkyl-7-hydroxy-4-
methylcoumarins following the procedure of S P Starkov, G
A Goncharenko and A I Panasencko, Zh. Obshch. Khim (1993),
63 (5), 1111-1115.

25 Other compounds of formula (I) were prepared
according to literature procedures as follows:

Bis-phenols

1,10-bis(2-hydroxyphenyl)decane and 1,10-bis(3-
hydroxyphenyl)decane

30 Antioxidants III. K Kakemi, T Arita, R Hori, and H
Takenaka Yakugaku Zasshi 86 (1966) 791-796

1,10-bis(4-hydroxyphenyl)decane

α,ω-di-p-hydroxyphenyl Alkanes

35 E M Richardson and E E Reid J. Am. Chem. Soc. (1940) 62
413-415

Fries transformation of condensates of sebacic acid

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- with phenols: 1,8-dibenzoyloctanes
J P Varma and J S Aggarwal J.Indian Chem.Soc. (1959)
36 41-45
- 5 Synthesis of α,ω -bis(p-hydroxyalkanes)
Y E Doroshenko and V A Sergeev
Zh. Organ. Khim. (1965) 1 (9) 1602-1604
- 10 1,12-bis(4-hydroxyphenyl)dodecane
Synthesis of α,ω -bis(p-hydroxyalkanes)
Y E Doroshenko and V A Sergeev Zh. Organ. Khim.
(1965) 1 (9) 1602-1604
- 15 1,14-bis(4-hydroxyphenyl)tetradecane
H Goldmann et al. J.Am.Chem.Soc. (1988) 110 (20)
6811-6817
- 15 1,10-bis(4-hydroxy-3-methylphenyl)decane
P Schlack and W Koller Ger. 1,086,711 Aug 11, 1960.
- 20 1,1-bis(2-hydroxyphenyl)decane
G Casiraghi et al. J. Chem. Soc. Perkin Trans 1
(1982), 3, 805-808.
- 20 Alkylphenols
The synthesis of aromatic hydroxyketones. I. ortho
and para-acylphenols with normal C₄-C₉ chains.
G Sandulesco and A Girard Bull.Soc.Chim.Fr. (1930)
47 (4) 1300-1314
- 25 Fungicidal activity and chemical constitution,
D Wood J.Chem.Soc. (1955), 4391-4393
- 30 Alkylation of phenol by 1-dodecane and 1-decanol. A
literature correction.
B Campbell, S Donald et al. Bull.Chem.Soc. Japan
(1990) 63 (12) 3665-3669 decylphenol and
dodecylphenol

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Cyclohexylphenols (ortho & para)

The direct alkylation of phenol by cyclohexene in the presence of boron trifluoride

H Lejebure and E Levas Compt. Rend. (1945) 220 782-784 and 826-827

H Lejebure and E Levas Compt. Rend. (1945) 221 301-303

Syntheses of α,ω -bis(2,4-dihydroxy) compounds.

Reaction of aliphatic dicarboxylic acids with resorcinol, J von Braun et al Ber. 74B 1772-1783 (1941).

Fries transformations of condensates of sebacic acid with phenols: 1,8-dibenzoyloctanes, J P Varma et al, J.Indian Chem.Soc. 36 41-45 (1959).

15 The Ca^{2+} - stimulated, Mg^{2+} - dependent, adenosine triphosphatase (Ca^{2+} -ATPase) located on plasma membranes extrudes Ca^{2+} against its electrochemical Ca^{2+} gradient. It has been reported that Ca^{2+} -ATPase not only plays a fundamental role in regulating the total cellular Ca^{2+} concentration but also modulates or mediates the effects of Ca^{2+} mobilising hormones and neurotransmitters. (See Pripic V, Green K C, Blackmore P R and Exton J H Vasopressin-, angiotensin II-, and α_1 -adrenergic -induced inhibition of Ca^{2+} -transportation by rat liver plasma membrane vesicles, (1984) J.Biol.Chem. 259, 1382-1385 and Rega A F, Garahan P J, (1986) The Ca Pump of Plasma membranes, CRC Pres Inc., Florida.

30 Natural and synthetic compounds of formula (I) were tested for their ability to influence human erythrocyte plasma membrane Ca^{2+} -ATPase. The enzyme Ca^{2+} -ATPase in the human red blood cell plasma membrane has previously been studied and its stimulation by calmodulin and activation by lipids and proteolysis and its primary structure have been published (see Carafoli E (1991) Calcium pump of the plasma membrane, Physiological Reviews 71, 129-153.

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The activity of compounds of formula (I) in the inhibition of Ca^{2+} -ATPase is shown in Table 1 and is interpreted graphically in figures 1, 2, 3 and 4.

5 Table 1 : Structures and IC_{50} values of natural and synthetic compounds of formula (I) in the inhibition of Ca^{2+} -ATPase (% inhibition was determined at a concentration of $100\mu\text{M}$).

	COMPOUND	SOURCE	INHIBITION OF Ca^{2+} -ATPase	
			PERCENT (%)	IC_{50} (μM)
10	2-octylphenol	b	100	32
	2-nonylphenol	b	95	30
	2-nonanoylphenol	b	32	>100
15	2-decylphenol	b	85	42
	nonylphenol (commercial)	a	100	27.5
	4-octylphenol	b	28	92
	4-tert-octylphenol	a	94	60
20	3-nonylphenol	b	69	64
	4-nonylphenol	b	22	114
	4-decylphenol	b	24	170
	2-cyclohexylphenol	h	28	186
	4-cyclohexylphenol	h	16	271
25	1,10-bis (2-hydroxyphenyl)- decane	b	100 ⁱ	7.6
	1,12-bis (2-hydroxyphenyl)- dodecane	e	100 ⁱ	8.3
30	1,14-bis (2-hydroxyphenyl)- tetradecane	e	84	28
35	1-(2-hydroxyphenyl)- -10-(4-hydroxyphenyl) decane	e	100 ^j	12
40	i - at $25\mu\text{M}$ j - at $50\mu\text{M}$ k - at $37\mu\text{M}$			

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	COMPOUND	SOURCE	INHIBITION OF Ca^{2+} -ATPase	
			PERCENT (%)	IC_{50} (μM)
5	1-(2-hydroxyphenyl) -12-(4-hydroxyphenyl) dodecane	e	99	18.8
	1-(2-hydroxyphenyl) -14-(4-hydroxyphenyl)- tetradecane	e	60	81
10	1,10-bis (4-hydroxyphenyl)- decane	b	52	93
15	1-(3,5-dihydroxy- phenyl)-14-(3,5- dihydroxy-4- methylphenyl)tetradecane (grebustol-A)	c	100 ^j	17
20	1,14-bis(3,5-dihydroxy- -4-methylphenyl)- tetradecane (striatol)	f	98	16
	striatol-B	f	100	40
	norstriatol-B	c	61 ^k	35
	5-nonylresorcinol	f	44	108
25	1,14-bis (4-hydroxyphenyl)- tetradecane	b	5	280
	1,14-bis(3,5-dihydroxy- phenyl)tetradecane (bisnorstriatol)	f	35	>100
30	1,14-bis(3,5-dihydroxy- phenyl)tetradec-Z-6-ene (grebustol-B)	c	90	50
35	ethyl 2,4- dihydroxy-6- nonylbenzoate	f	73	62
	ethyl 3,5-dibromo- 2,4-dihydroxy-6- nonylbenzoate	f	95	44
	grevillol	c	44	143
40	5-decylresorcinol	f	30	135
	ethyl 2,4- dihydroxy-6- decylbenzoate	f	60	85
45	ethyl 3,5-dibromo-2,4- dihydroxy-6- decylbenzoate	f	73	69

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	COMPOUND	SOURCE	INHIBITION OF Ca^{2+} -ATPase	
			PERCENT (%)	IC_{50} (μM)
5	2- <u>E</u> , <u>E</u> -farnesyl-5-methyl-resorcinol (grifolin)	d	100	22.5
	4- <u>E</u> , <u>E</u> -farnesyl-5-methyl-resorcinol (neogrifolin)	d	100	23.3
10	4-dodecylresorcinol	a	68	69
	4-hexylresorcinol	a	17	259
	2,4,6-tri-tert-butylphenol	a	79	15
15	3,5-di-tert-butylcatechol	a	76	84
	2,6-di-tert-butyl-4-methylphenol (BHT)	a	69	66
	2,6-di- <u>tert</u> -butylphenol	a	61	79
20	2,6-di-tert-butyl-4-methoxyphenol (BHA)	a	44	>400
	2,2'-methylenebis(4-methyl-6-tert-butylphenol)	g	74	45

25 The activity of the synthetic and natural compounds in the inhibition of Ca^{2+} -ATPase in the presence (+CaM) and in the absence (-CaM) of Calmodulin (CaM) is shown in table 1A below and is interpreted graphically in the figures 5, 6, 7, 8, 9.

30 Table 1A

	SOURCE	COMPOUND	IC_{50}	
			(-CaM)	(+CaM)
	b	2-nonylphenol	36	40
	b	2-methyl-6-nonylphenol	-	-
	b	3-methyl-6-nonylphenol	52	60
35	b	4-methyl-6-nonylphenol	80	75
	b	2-bromo-6-nonylphenol	-	-
	b	4-bromo-6-nonylphenol	52	55

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SOURCE	COMPOUND	IC ₅₀	
		(- CaM)	(+CaM)
5	b 2,4-dibromo-6-nonylphenol	-	-
	b 2-nitro-6-nonylphenol	-	-
	b 4-nitro-6-nonylphenol	80	70
	b 2-bromo-4-nitro-6-nonylphenol	-	70
	b 4-bromo-2-nitro-6-nonylphenol	-	65.5
10	b 4-nonylresorcinol	125	ND
	a 4-dodecylresorcinol	80	100
	b 2-bromo-6-nonylresorcinol	400	400
	b 4-bromo-6-nonylresorcinol	400	400
	b 2,4-dibromo-6-nonylresorcinol	-	-
15	b 1,8-bis(2-hydroxyphenyl) octane	24	24
	b 1,9-bis(2-hydroxyphenyl) nonane	13.5	14
	b 1,10-bis(2-hydroxyphenyl) decane	8.4	9.0
	e 1,12-bis(2-hydroxyphenyl) dodecane	12.5	13
	b 1,10-bis(2-hydroxy-3- methylphenyl)-decane	50	55
	b 1,10-bis(2-hydroxy-4- methylphenyl)-decane	29	29
	b 1,10-bis(2-hydroxy-5- methylphenyl)-decane	22	20.5
	b 1,8-bis(2,4-dihydroxyphenyl) octane	50	48

SOURCE	COMPOUND	IC ₅₀	
		(-CaM)	(+CaM)
b	1,10-bis(2,4-dihydroxyphenyl)decane	10	16
e	1,11-bis(2,4-dihydroxyphenyl)undecane	25	21.7
e	1,12-bis(2,4-dihydroxyphenyl)dodecane	12	20
e	1,10-bis(2,4-dihydroxy-3-methylphenyl)decane	100	100
5 b	1,10-bis(3-hydroxyphenyl)decane	30	32
e	1,10-bis(3-hydroxy-4-methylphenyl)-decane	51	48
b	1,10-bis(4-hydroxy-3-methylphenyl)-decane	100	94
b	1,1-bis(2-hydroxyphenyl)decane	74	68
e	1,10-bis(2-hydroxy-1-naphthyl)decane	92	90
10 e	6-dodecyl-7-hydroxy-4-methylcoumarin	NA	NA
b	2-methyl-5-nonyl-resorcinol	NA	NA
i	5,7,2',6'-tetrahydroxy-8-lavandulylflavanone	20	ND
i	5,7,6'-trihydroxy-8-lavandulylflavanone	26.5	ND
i	5,2',6'-trihydroxy-8-lavandulyl-7-methoxyflavanone	45.3	ND

15 NA: no inhibitory activity; ND: not determined

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Source

- a = commercially available from Aldrich, Milwaukee, WI, USA
- b = synthetic preparation from literature procedure
- 5 c = extracted from Grevillea
- d = natural products provided by Prof Shibata [Misasa H, Matsue Y, Vehara H, Tanaka H, Ishihara M, Shibata H, (1992) Tyrosinase inhibitors from *Albatrellus confluens*, Biosci.Biotech.Biochem., 56 1660-1661]
- 10 e = novel compounds of formula (II) according to the present invention
- f = natural and synthetic substances provided by Prof W C Taylor [see references, page 9, lines 25-30 also Cleaver L, Croft J A, Ritchie E and Taylor W C (1976) Chemical studies of the Proteaceae IX Aust.J.Chem., 29, 1989-2001]
- 15 g = commercially available from Merck, Darmstadt, Germany
- 20 h = synthetic substance provided by Prof Kanzo Sakata, Shizuoka University, Shizuoka Japan [see reference for cyclohexylphenols (ortho & para) on page 17]
- 25 i = natural substances provided by Assoc. Prof. C Chaichantipyuth, Chulalongkorn University of Thailand (see Ruangrunsi N et al, Photochemistry, 31, 999-1001, 1992 and Iinuma M et al, Photochemistry, 33, 203-208, 1993.
- 30 The potency of a compound for Ca^{2+} -ATPase inhibition is characterised by either its % inhibition at a single dose or more informatively as its IC_{50} value from the dose-response curve. The most potent compounds have, therefore, curves located at the left hand side of the
- 35 graphs. From figure 2, it can be seen that 1,10-bis(2-hydroxyphenyl)decane and, to a lesser extent, 1,12-bis(2-hydroxyphenyl) dodecane have the strongest Ca^{2+} -ATPase inhibition among the synthesised phenolic compounds. The

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inhibitory activity is two-fold higher than that of the natural product 1,14-bis(3,5-dihydroxy-4-methylphenyl)tetradecane (striatol) and is superior to the 2-alkylphenol series. Of the alkylphenols, the maximum activity appeared with 2-nonylphenol ($IC_{50} = 30\mu m$). The results of nonylphenols and octylphenols showed that the potency order of alkylphenols was 2-alkylphenols, 4-branched alkylphenols, and 4-alkylphenols. Similarly, 4-dodecylresorcinol was the most potent among the alkylresorcinols tested and grifolin and neogrifolin the most potent among the alkyl and alkenyl resorcinols tested.

The results also show the importance of substitution at the 2- position of the phenol ring for strong Ca^{2+} -ATPase inhibition. The results indicate that there is a significant difference in potency between ortho- and para- substituted compounds, whereas the activity of the meta- substituted compound is intermediate.

The results further indicate a structural preference for the inhibitory potency of the synthetic compounds and an optimal methylene chain length for the bis compounds is ten. 1,10-bis(2-hydroxyphenyl)decane and 1,10-bis(2,4-dihydroxyphenyl)decane to lesser extent are the most potent inhibitors of plasma Ca^{2+} -ATPase among the synthesised substances.

In the presence or absence of Calmodulin (CaM), in general, there is no significant difference in the inhibitory activity of the synthesised compounds (refer to table 1A). However, it is observed that at higher concentration of the test compounds, there was slightly higher inhibition of plasma membrane Ca^{2+} -ATPase than in the absence of CaM (refer to figures 5,7,8,9). This would indicate that the compounds may also inhibit CaM stimulant activity.

^{45}Ca efflux from cultured vascular smooth muscle cells consists of two major mechanisms; one is dependent on extracellular sodium, mediated by the Na^{+} - Ca^{2+} exchanger (Na^{+} - dependent Ca^{2+} efflux), and the other is

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independent of extracellular sodium but is mediated by the Ca^{2+} pump (Na^{+} - independent Ca^{2+} efflux). The effect of striatol on the efflux of calcium ($^{45}\text{Ca}^{2+}$) from rat thoracic aorta smooth muscle cells in culture was studied. $^{45}\text{Ca}^{2+}$ efflux is a measure of plasma membrane Ca^{2+} -ATPase (plus Na^{+} : Ca^{2+} exchange) in intact (whole) cells. The results are as follows:

	20 μM 5 min	-	partial inhibition
	20 μM 10 min	-	partial inhibition
10	50 μM 30 min	-	complete inhibition

Compounds of formula (I) show significant inhibitory activity against plasma membrane Ca^{2+} -ATPase and typically would be suitable for use in the treatment of cardiovascular disease. These compounds would be useful by their action on Ca^{2+} -ATPase enzymes in general or on Na^{+} , K^{+} -ATPase enzyme. In particular, compounds of formula (I) may be suitable for the treatment or prophylaxis of chronic heart failure, angina, hypertension or arrhythmia.

Accordingly, in another aspect, the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable derivative thereof in the manufacture of a medicament for the treatment or prophylaxis of cardiovascular disease.

The effective amount of the active compound required for use in the above conditions will vary both with the route of administration, the condition under treatment and the host undergoing treatment, and is ultimately at the discretion of the physician. In the above mentioned treatments, it is preferable to present the active compound as a pharmaceutical formulation. A pharmaceutical formulation of the present invention comprises the active compound together with one or more pharmaceutically acceptable carriers and optionally any other therapeutic ingredient. The formulation may conveniently be prepared in unit dosage form and may be prepared according to conventional pharmaceutical techniques. Additionally, the formulations may include

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one or more accessory ingredients, such as diluents, buffers, flavouring agents, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives and the like.

5 A typical tablet formulation comprises 1-50mg of the active constituent, 50-200mg of lactose, 7-28mg of maize starch and 0.25-1mg of magnesium stearate. Preferably, the tablet formulation comprises 1-50mg of the active constituent, about 97mg of lactose, about 14mg of maize
10 starch and about 0.5mg of magnesium stearate.

Compounds of formula (I) may also be useful for the treatment of ulcers (peptic ulcers) through H^+ , K^+ -ATPase inhibition (the proton pump in gastric parietal cells) or may act as depigmentation, antidiabetic,
15 antithrombolytic, antiarteriosclerotic, antioxidant, anticancer, antiinflammatory or antiviral agents.

EXPERIMENTAL - SYNTHESSES

Instrumentation

Thin layer chromatographic plates were visualised by
20 a UVSL-58 mineral-light lamp, multiband UV-254/366nm. Precoated Si gel plates (Merck, Art. 5554) were utilised.

Analytical high-performance liquid chromatography were obtained with a Beckman 110B solvent module with a PC-3800 controller.

25 Detection was achieved with a Spectra-Physics Spectra 100 variable wavelength detector.

Analytical column was an Activon exsil 100/10 ODS, 250 x 4.6mm i.d. (reversed-phase C_{18}).

Preparative short column was 70 x 65 mm i.d.
30 connected with an one litre glass bell jar.

1H - and ^{13}C -NMR spectra were obtained on a Varian Gemini 300, and Joel FX90Q (for grevillol) spectrometer using $CDCl_3$ as solvent and referencing to tetramethylsilane.

35 UV spectra were measured on a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer.

Chemical ionisation and electron impact mass spectra were performed on a Finnigan TSQ46 spectrometer. All

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chemical ionisation spectra were performed using methane as reagent gas.

Materials

Ethyl acetate and petroleum (70-75°C) were distilled prior to use. Thionyl chloride was distilled at 75 - 80°C

Dichloromethane, chloroform, methanol were HPLC grade. Merck silica gel 60H (Art. 7736) was used for preparative TLC and short column chromatography.

3,4,5-trimethoxybenzaldehyde, n-butyllithium, undecandioic acid, 1,6-dibromohexane, 1,8-dibromooctane, 1,10-dibromodecane, 1,12-dibromododecane, 2-methylresorcinol, 1,10-decanedicarboxylic acid and 1,12-dodecanedicarboxylic acid were purchased from Aldrich Chemical company.

I. Synthesis of alkylphenols

1. Preparation of 2- and 4-octylphenol

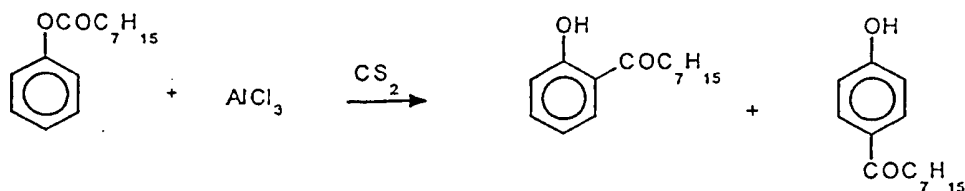
1.1 Preparation of phenyl octanoate

The ester was prepared by slowly adding thionyl chloride (10ml) to a mixture of pure phenol (6.5g) and octanoic acid (10g). The reaction mixture was warmed to drive off the sulfur dioxide and hydrogen chloride gases. The crude mixture was distilled at 95-100°C/5mmHg.

Phenyl nonanoate and phenyl decanoate were prepared similarly. The esters were distilled at 105-110°C and 120-125 °C/5mmHg respectively.

The yield of the esters was 81-84%.

1.2. Preparation of 2- and 4-octanoylphenol



Anhydrous aluminium chloride (7g) and carbon disulfide (10ml) were placed in a three-necked round bottom flask fitted with a reflux condenser, a 50ml-dropping funnel and a large magnetic stirrer. Phenyl octanoate (10g) was slowly added to the stirred suspension

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through the dropping funnel. When all the ester was added, the mixture was further heated to gentle refluxing on a steam bath until the evolution of hydrogen chloride had almost ceased (about 1/2hr). The reflux condenser was turned downward and carbon disulfide was distilled. The steam bath was replaced by an oil bath which was heated to 140°C and maintained at 140-150°C for one hour. The mixture thickened and finally congealed to a brown resinous mass. The solid was allowed to cool and the aluminium complex was decomposed by first slowly adding a mixture of concentrated hydrochloric acid(6ml) with water(6ml) and then water(10ml). The mixture was left overnight and large solid portion (mainly 4-octanoylphenol) on the surface was collected. The liquid portion was extracted with ethyl acetate. The extract was combined with the solid portion and the resulting mixture was dried with anhydrous sodium sulfate, filtered and evaporated to obtain the crude mixture. The products, 2- and 4-octanoylphenols, were separated by short column vacuum chromatography based on the difference in their polarity.

The yield was 29-34% of 2-octanoylphenol and 46-50% of 4-octanoylphenol. The products were then characterised by NMR and CI-MS analysis (refer to tables 3 & 4).

Separation of the products using short column vacuum chromatography

The crude mixture was dissolved firstly in dichloromethane (one-part) then petroleum (four-parts). The resulting mixture (25ml) was loaded onto a (70mm diam.x 30mm) silica gel bed under vacuum and continuously washed with petroleum (40ml).

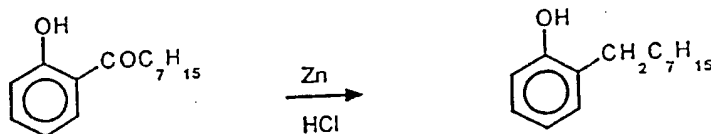
Eluent solvent mixtures used to isolate the products were a stepwise solvent gradient from petroleum spirit/ethyl acetate 9:1 to 1:1 (20 fractions).

Fractions were examined under UV light by TLC to determine the degree of separation between products. NMR and CI-MS analysis were performed on fractions #5-10

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(identified as 2-octanoylphenol) and #14-20 (identified as 4-octanoylphenol).

1.3. Preparation of 2- and 4-octylphenol



5 Amalgamated zinc(10g) was placed in a 100ml round-bottom flask fitted with a stirrer and a reflux condenser. A mixture of acetic acid(10ml) and concentrated hydrochloric acid(10ml) was added and then a solution of 2-octanoylphenol (2g) (or 4-octanoylphenol)
10 in acetic acid(5ml). The mixture was agitated and refluxed for 2 days. Aqueous 20% w/v NaCl solution(20ml) was added and the mixture was extracted with petroleum(20ml). The petroleum portion was dried with anhydrous sodium sulfate, filtered and purified by short
15 column vacuum chromatography.

A yield of 82-84% was obtained.

The column chromatographic method used was the same as described above.

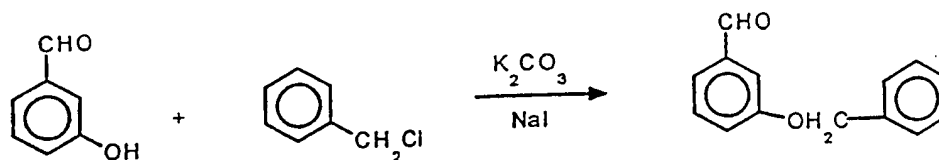
20 The products were characterised by NMR and CI-MS analysis (Refer to tables 3 & 4).

Note: 1. The zinc was amalgamated in the reaction flask by covering it with a solution of mercuric chloride(0.2g) in water(15ml) and occasionally agitated over 30 minutes. The solution was poured off and the zinc was rinsed once
25 with water.

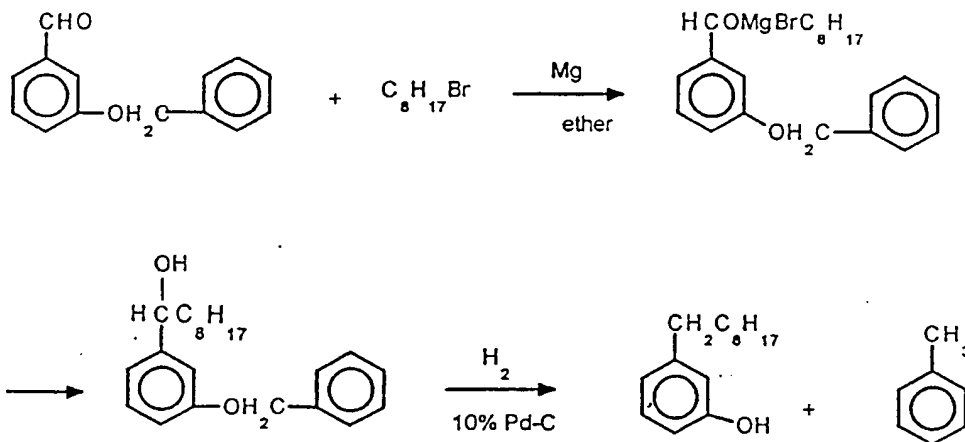
2. Aqueous 20% w/v NaCl was added to increase the ionic strength of the aqueous phase so that the octylphenol could be extracted into the organic phase.

30 Nonyl- and decylphenols were prepared as described for octylphenol. The products at each stage were also characterised by NMR and CI-MS analysis.(Refer to tables 3 & 4).

2. Preparation of 3-nonylphenol

2.1 Preparation of 3-benzyloxybenzaldehyde

In a 100ml round-bottom flask were placed 3-hydroxybenzaldehyde (5g), benzyl chloride(6g), sodium iodide(8g) and potassium carbonate(10g). The reaction mixture was sealed and stirred for one day. The crude product was extracted with ethyl acetate(20ml). The organic portion was washed twice with distilled water(20ml) to remove all aqueous soluble materials. The ethyl acetate portion was dried with anhydrous sodium sulfate and evaporated to obtain the crude solid. The solid was purified by the column chromatographic method described for octylphenol. The product was identified by NMR and CI-MS analysis (Refer to tables 3 & 4).

2.2 Preparation of 3-nonylphenol

Grignard reagent was prepared from magnesium(248mg) and 1-bromooctane(1.8g) in dry diethyl ether(5ml). A tiny amount of solid iodine was added to initiate the reaction and ether(10ml) was further added. The reaction was stirred until all magnesium dissolved and 3-benzyloxybenzaldehyde(10g) was then added to the reaction mixture. The reaction was refluxed at room temperature for 4 hours. Ice water was then added. The organic

- 32 -

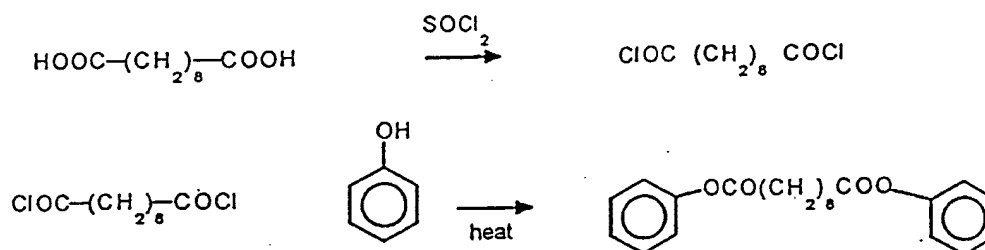
phase was separated and washed with 1.5M sulfuric acid (2x25ml), 10% potassium carbonate (2x25ml), water (20ml), 1M hydrochloric acid (20ml) in saturated sodium chloride, dried with anhydrous sodium sulfate and evaporated to obtain the product (79% of yield).

A portion of the product(500mg) was hydrogenated by catalytic reduction over 10% palladium-charcoal(130mg) in ethyl acetate (50ml) containing concentrated sulfuric acid(10 drops). The reaction mixture was stirred at room temperature for one day. The mixture was then filtered, evaporated and purified by the column chromatographic method.

The product was identified by NMR and CI-MS analysis (Refer to tables 3 & 4).

II. Synthesis of α,ω -bis(hydroxyphenyl)alkanes.

1. Preparation of bisphenyl decanedioate



The methods of preparation, isolation and purification were similar to those described for octylphenol except that the preparation of esters was done in two separate steps, as shown above, instead of one as described for phenyl octanoate.

Firstly, the acid dichloride was prepared by refluxing the mixture of dicarboxylic acid(5g) and thionyl chloride(20ml) at 60-70°C for two hours. Thionyl chloride was then removed by evaporation and the remaining product was dissolved in toluene(5ml). The toluene was evaporated to remove all traces of thionyl chloride.

Secondly, the phenol(2x moles of acid dichloride) was added to the acid dichloride. The mixture was warmed to drive off hydrogen chloride gas. The product

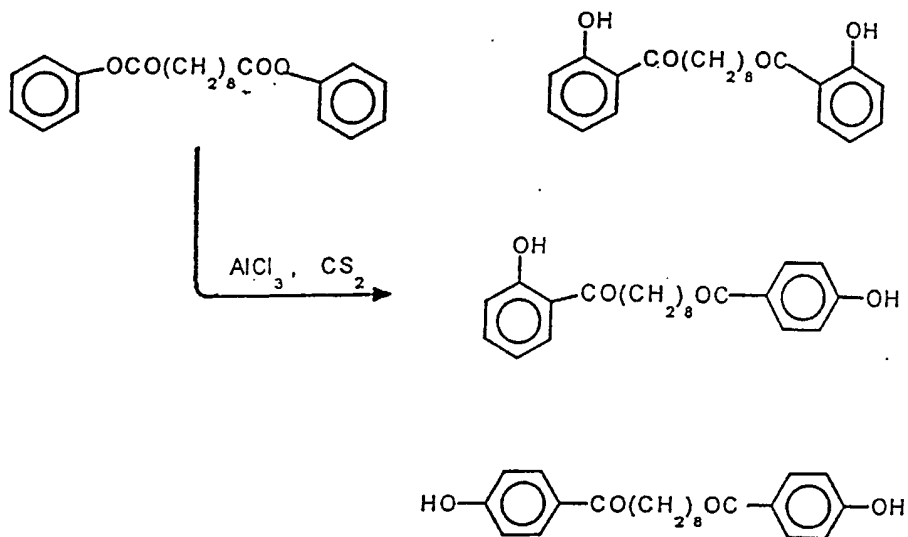
- 33 -

solidified on cooling and was purified by the column chromatographic method.

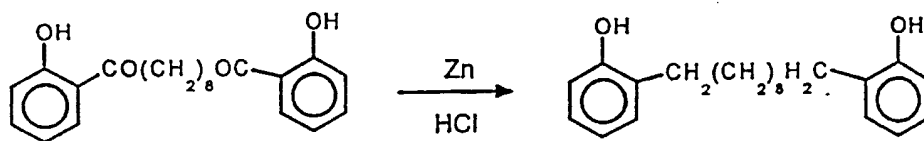
The yield of the esters was 96-98%

2. Preparation of 1,10-bis(hydroxyphenyl)decane-

5 1,10-diones



3. Preparation of 1,10-bis(2-hydroxyphenyl)decane



The product was characterised by NMR and CI-MS analysis

(Refer to NMR and CI-MS tables 3 & 4).

10 III. Synthesis of α,ω -bis[2-hydroxy-(3-,4- or 5-methyl)phenyl]alkanes

These were prepared according to K Kakemi et al. in Antioxidants III. K Kakemi, Y Arita, R Hori and H Takenaka. Yakugaku Zasshi 86, 791-796 (1966) as follows:

15 Aliphatic dicarboxylic acid chloride (1 mole) [for example sebacoyl chloride] was added to a solution of a phenol (2.2 moles) in tetrachloroethane containing

- 34 -

anhydrous AlCl_3 (2.5 moles). The mixture was stirred for 5-6 hrs at 70-80°C. The product was decomposed with ice-water and concentrated HCl (1:1). The organic layer was separated and concentrated under reduced pressure. The residue was extracted with ethyl acetate and washed with water twice. After removal of the solvent the product was subjected to gradient chromatography to isolate the corresponding diketones which on Clemmensen reduction gave the title compounds.

The products were recrystallised from petroleum or petroleum - ethyl acetate to give colourless crystalline solids.

The procedure was similarly applied to prepare bis(2-hydroxy-1-naphthyl)decane.

IV. 1,10-Bis(4-hydroxy-3-methylphenyl)decane

This was prepared by the method reported by Schlack and Koller in Aromatic aliphatic diketones. P Schlack and W Koller. Ger. 1,086,711 Aug. 11, 1960. The title compound was prepared by treatment of ortho-cresol (2.2 moles) with sebacic acid (1 mole) in the presence of polyphosphoric acid (3 moles). The mixture was stirred for 4 hrs at 80°C and poured to ice-water after cooling. The precipitant was filtered, dissolved in ethyl acetate and washed with water three times. The solvent was evaporated to yield a crude 1,10-bis(4-hydroxy-3-methylphenyl)decane-1,10-dione which was then subjected to Clemmensen reduction to give the title compound.

The product was recrystallised to yield a colourless crystalline white solid.

V. Synthesis of α,ω -bis(2,4-dihydroxy-(3-methyl)phenyl)alkanes

These were prepared according the literature procedure in Reactions of aliphatic dicarboxylic acids with resorcinol. J von Braun, E Anton and F Meyer. Ber. 74B, 1772-1783 (1941) as follows:

The aliphatic dicarboxylic acid (1 mole) was heated with anhydrous ZnCl_2 (2 moles) at 140°C followed by added

- 35 -

resorcinols (10 moles) in portions. Stirring of the mixture was maintained for 4-5 hrs at 140-160°C (except that for

2-methylresorcinol which was stirred at 170°C). The products were decomposed with ice-water. The solid was collected, washed with 10% Na₂CO₃ and then with water under vacuum.

The crude products were subjected to Clemmensen reduction to yield the title compounds and were recrystallised from ethanol - water to give colourless crystalline solids.

VI. Synthesis of 1,10-bis[3-hydroxy-4-methylphenyl]decane.

1,10-Bis(4-methylphenyl)decane-1,10-dione was prepared according to (III) above. The diketone dissolved in concentrated sulfuric acid were gradually added to the mixture of fuming nitric acid and concentrated sulfuric acid (2:1 in volume) at -5°C. The mixture was stirred at 0°C for 30mins and poured in to ice-water. The precipitate was filtered off and recrystallised from ethyl acetate to give 1,10-bis(3-nitro-4-methylphenyl)decane-1,10-diones which then underwent reduction with SnCl₂.2H₂O in the presence of concentrated HCl at elevated temperature of 90°C for 30mins. The precipitate that formed on cooling was collected, washed with HCl and dissolved in dilute NaOH. The solid precipitating on the addition of dilute HCl was collected and recrystallised from ethanol to yield 1,10-bis(3-amino-4-methylphenyl)decane-1,10-dione.

This product was diazotised with aq. NaNO₂ in 3M H₂SO₄ at 5°C. The mixture was then hydrolysed with 10% H₂SO₄ at 160°C. The resulting solution was extracted with ethyl acetate and the solvent was evaporated to yield 1,10-(bis(3-hydroxy-4-methylphenyl)decane-1,10-dione which on Clemmensen reduction and gave the title compound which was recrystallised from petroleum - dichloromethane.

VII. Synthesis of α,ω -bis(3,5-dihydroxy-4-

- 36 -

methylphenyl)alkanes.

a. 3,5-dimethoxy-4-methylbenzaldehyde was prepared by a procedure published in Regioselective reduction alkylation of 3,4,5-trimethoxy-benzaldehydes and dimethylacetal. U Azzena, S Cossu, T Denurra, G Melloni and A M Piroddi. Synthesis 1990, 313 and Regioselective reduction electrophilic substitution of derivatives of 3,4,5-trimethoxybenzaldehyde. U Azzena, G Melloni, A M Piroddi, E Azara, S Contini and E Fenude.

10 J. Org. Chem. 57, 3101-3106 (1992).

The diacetal intermediate was distilled at 130°C / 0.8 mmHg and 3,5-dimethoxy-4-methylbenzaldehyde (compound A) was recrystallised from petroleum.

b. Preparation of α -N,N-dimethylamino- α -cyano-(3,5-dimethoxy-4-methyl)benzylidene (Compound B) (see Benzylolation and related alkylation of α -dimethylamino-phenylacetonitrile by mean of alkali. C R Hauser, H M Taylor and T G Ledford. J. Am. Chem. Soc. 82, 1786 (1960).

20 To a stirred solution of sodium bisulfite (1 mole) in 400 ml of water was added compound A (1 mole) in methanol followed by anhydrous dimethylamine (1.2 moles) in aq. methanol (80%). The reaction mixture was cooled prior the addition of aq. sodium cyanide (1.2 moles). The mixture was stirred at room temperature for 20 hrs and diethyl ether (50 ml) was then added. The ethereal layer was washed with water twice and evaporated to yield the product Compound B (> 90%) which was sufficiently pure for the next reaction.

30 c. Preparation of α,ω -bis(3,5-dimethoxy-4-methylphenyl)alkane- α,ω -diones (see An efficient method for synthesis of symmetrical diketones via reaction of α -amino- α -arylacetonitriles with alkyl dibromides. K Takahashi, M Watsuzaki, K Ogura and H Iida. J. Org. Chem. 48, 1909-1912 (1983).

35 Under dry nitrogen diisopropylamine (1,5 ml, 9 mmol) dissolved in a mixture of 5 ml each of dry THF and HMPA was treated with n-butyllithium (4 ml of 2.5 M solution

- 37 -

in hexane, 10 mmol) at -78°C. Compound B (8 mmol) dissolved in THF (2 ml) was added and the reaction mixture was stirred for 15mins at -78°C and for 1hr at 0°C. To the mixture cooled to -20°C was added α,ω -dibromoalkanes (4 mmol) dropwise. After the mixture was stirred for 20mins at -20°C, the stirring was continue overnight at room temperature.

The reaction mixture was poured into ice-water and extracted with diethyl ether (3x50 ml). The combined ethereal layers was washed with brine and concentrated under reduced pressure. The residue dissolved in a solution of 3 ml each of THF and 30% aq. oxalic acid was refluxed for 90mins then extracted with diethyl ether. After the solvent was evaporated the product was recrystallised from ethanol to give the diketones (Compounds C).

d. Preparation of α,ω -bis(3,5-dihydroxy-4-methylphenyl)alkanes.

The Compounds C were subjected to Clemmensen reduction and then demethylation with 47% HBr in the presence of acetic acid at 130°C for 10 hrs. The product was subjected to gradient chromatography and recrystallised from benzene to give a colourless crystalline solid.

1,1-Bis(2-hydroxyphenyl)decane was prepared according to the literature procedure of G Casiraghi et al. in Regiospecificity in reactions of metal phenoxides. Synthesis of 2,2-alkylidenebisphenols. G Casiraghi, G Casnati, A Pochini and R Ungaro. J. Chem. Soc., Perkin Trans.1 (1982), 3, 805-808. The product was distilled at 210°C/0.4mmHg.

6-dodecyl-7-hydroxy-4-methylcoumarin was prepared according to the literature procedure of S P Starkov et al. in Condensation of ethyl acetoacetate and ethyl benzoylacetate with 4-alkylresorcinols in the presence of boron trifluoride etherate. S P Starkov, G A Goncharenko and A I Panasenko. Zh. Obshch. Khim. (1993), 63(5), 1111-1115.

- 38 -

The product was recrystallised from ethanol.

- Bromination of 2-nonylphenol was carried out according to D E Rearson et al. in The ortho bromination of phenols. D E Rearson, R D Wysong and C V Breder. J. Org. Chem. (1967), 35(19), 3221-3231.

Nitration of 2-nonylphenol was carried out according to D S Ross et al. in Catalysis of aromatic nitration by the lower oxides of nitrogen. D S Ross, G P Hum and W G Blucher. J. Chem. Soc., Chem. Comm. 1980, 532-533.

- 10 Bromination of 4-dodecylresorcinol was carried out according to E Kiehlmann and R W Lauener in Bromophloroglucinols and their methyl ethers. E Kiehlmann and R W Lauener. Can. J. Chem. (1989), 67, 335-344.

- 15 4-bromo-6-dodecylresorcinol was prepared according to procedure 8 above in The ortho bromination of phenols. D E Rearson, R D Wysong and C V Breder. J. Org. Chem. (1967), 35(19), 3221-3231.

* Clemmensen reduction was carried out as follows:

- 20 The ketone compounds dissolved in toluene were added to the mixture of concentrated HCl and acetic acid (1:1) containing amalgamated zinc. The reaction mixture was refluxed for 10hrs with vigorous stirring or stirred at room temperature for 2 days.

Table 2: Synthetic phenolic products

COMPOUND (No)	%YIELD
2-octylphenol (1) 2-decylphenol (3)	19.8 - 23.3
4-octylphenol (4) m.p. 40-42°C 4-nonylphenol (5) m.p. 27-38°C 4-decylphenol (6) m.p. 53-55°C	31.5 - 34.2
3-nonylphenol (7)	10.0
1,14-bis(2-hydroxyphenyl)tetradecane (14)	20.8 - 23.3
1-(2-hydroxyphenyl)-10-(4-hydroxyphenyl)decane (9) 1-(2-hydroxyphenyl)-12-(4-hydroxyphenyl)dodecane (12) 1-(2-hydroxyphenyl)-14-(4-hydroxyphenyl)tetradecane (15)	22.5 - 25
1,10-bis(4-hydroxyphenyl)decane (10) 1,12-bis(4-hydroxyphenyl)dodecane (13) 1,14-bis(4-hydroxyphenyl)tetradecane (16)	6.7 - 8.3

Table 2: continued

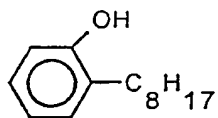
	COMPOUNDS	MELTING POINT °C	% YIELD
	2-nonylphenol (2)	liq.	58-60
	2-methyl-6-nonylphenol	liq.	56
5	3-methyl-6-nonylphenol	liq.	52
	4-methyl-6-nonylphenol	liq.	50
	2-bromo-6-nonylphenol	liq.	40
	4-bromo-6-nonylphenol	45-47	85
	2,4-dibromo-6-nonylphenol	liq.	>95
10	2-nitro-6-nonylphenol	liq.	45
	4-nitro-6-nonylphenol	liq.	38
	2-bromo-4-nitro-6-nonylphenol	64-65	>90 ^a
	4-bromo-2-nitro-6-nonylphenol	61-63	
	4-nonylresorcinol	70-71	80
15	2-bromo-6-dodecylresorcinol	68-69	70 ^b
	4-bromo-6-dodecylresorcinol	61-62	82
	2,4-dibromo-6-dodecylresorcinol	58-59	>95
	6-dodecyl-7-hydroxy-4-methyl- coumarin	135-136	75
20	1,8-bis(2-hydroxyphenyl)octane	74	40-45
	1,9-bis(2-hydroxyphenyl)nonane	64-65	
	1,10-bis(2-hydroxyphenyl)decane (8)	81-82	
	1,12-bis(2-hydroxyphenyl)- dodecane(11)	88.5	
25	1,10-bis(2-hydroxy-3- methylphenyl)decane	82-83	38

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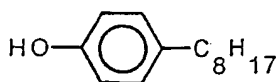
	COMPOUNDS	MELTING POINT °C	% YIELD
	1,10-bis(2-hydroxy-4-methylphenyl)decane	102-103	40
	1,10-bis(2-hydroxy-5-methylphenyl)decane	86	25
5	1,8-bis(2,4-dihydroxyphenyl)-octane	167-168	75-80
	1,10-bis(2,4-dihydroxyphenyl)-decane	155-156	
10	1,11-bis(2,4-dihydroxyphenyl)-undecane	136-137	
	1,12-bis(2,4-dihydroxyphenyl)-dodecane	146-147	
	1,10-bis(2,4-dihydroxy-3-methylphenyl)decane	138-140	70
15	1,10-bis(3-hydroxyphenyl)decane	71-73	18-20
	1,10-bis(3-hydroxy-4-methylphenyl)decane	100-102	
	1,10-bis(4-hydroxy-3-methylphenyl)decane	82-83	78
20	1,1-bis(2-hydroxyphenyl)decane	liq.	70
	1,10-bis(2-hydroxy-1-naphthyl)-decane	101-103	40
	2-methyl-5-nonyl-resorcinol	91-92	70
25	1,8-bis(3,5-dihydroxy-4-methylphenyl)octane	173-175	65-70
	1,10-bis(3,5-dihydroxy-4-methylphenyl)decane	139-140	

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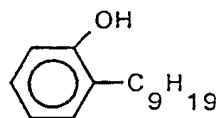
COMPOUNDS	MELTING POINT °C	% YIELD
1,12-bis(3,5-dihydroxy-4-methylphenyl)dodecane	123-124	
1,14-bis(3,5-dihydroxy-4-methylphenyl)tetradecane	130-131	



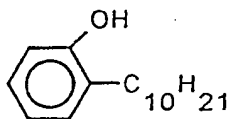
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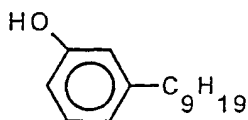
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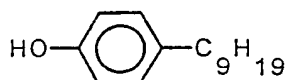
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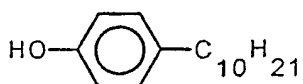
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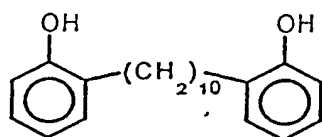
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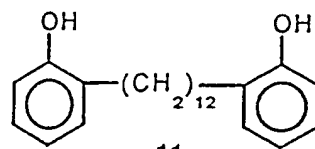
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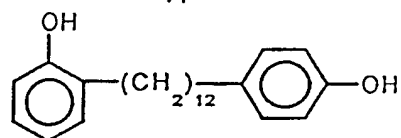
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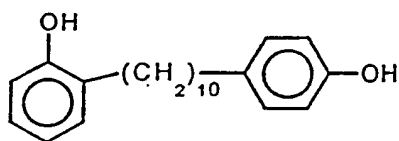
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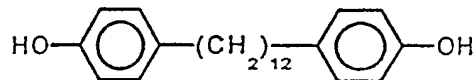
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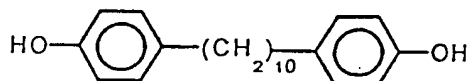
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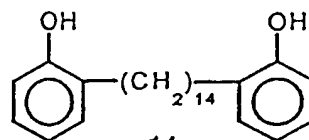
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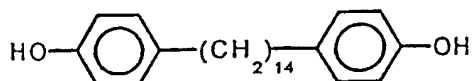
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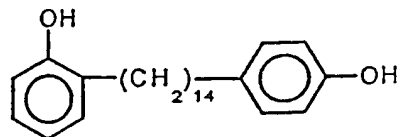
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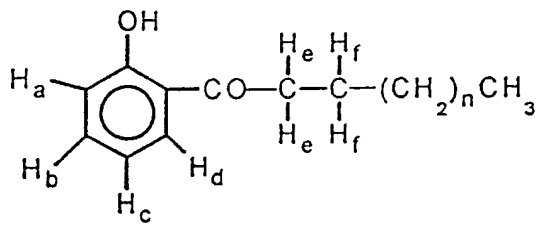
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Structures of synthetic alkylphenols and
 α,ω -bis(hydroxyphenyl)alkanes.

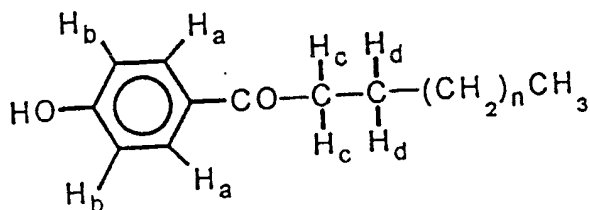
Table 3: ^1H -NMR spectra of synthesised products

s:singlet, d:doublet, t:triplet, q:quintet, m:multiplet, b:broad

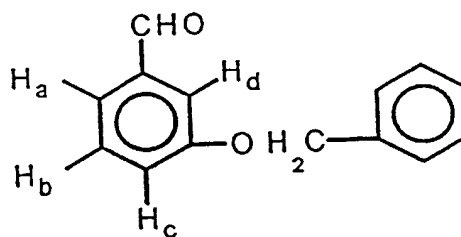
 ^1H -NMR spectra of the intermediate products

Compound	δ in ppm	No of protons	Multi- plicity	Assignment
<u>2-alkanoylphenols</u> 				
2-octanoylphenol (n=4)	0.88	3	t(b)	-CH ₃
	1.3	8	m(b)	-CH ₂ -
2-nonanoylphenol (n=5)	1.3	10	m(b)	-CH ₂ -
2-decanoylphenol (n=6)	1.3	12	m(b)	-CH ₂ -
	1.7	2	q(b)	Hf
	2.9	2	t	He
	6.88	1	dt	Hc
	6.98	1	dd	Ha
	7.45	1	dt	Hb
	7.77	1	dd	Hd

$J_{ab} = 8 \text{ Hz}$, $J_{ac} = 2 \text{ Hz}$, $J_{bc} = 8 \text{ Hz}$, $J_{bd} = 2 \text{ Hz}$, $J_{cd} = 8 \text{ Hz}$

4-alkanoylphenols

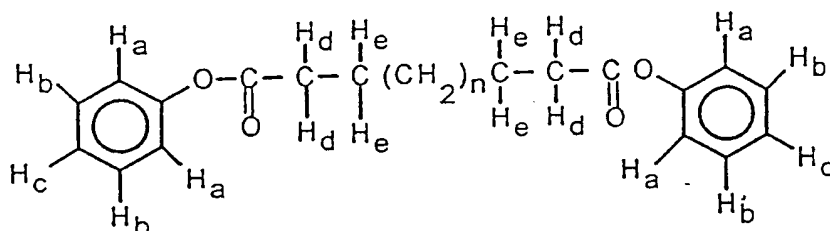
4-octanoylphenol (n=4)	0.88	3	t(b)	-CH ₃
	1.30	8	m(b)	-CH ₂ -
4-nonanoylphenol (n=5)	1.30	10	m(b)	-CH ₂ -
4-decanoylphenol (n=6)	1.30	12	m(b)	-CH ₂ -
	1.70	2	q(b)	Hd
	2.90	2	t	Hc
	6.89	2	d	Hb
	7.80	2	d	Ha

 $J_{ab} = 8 \text{ Hz}$ 3-benzyloxybenzaldehyde

5.11	2	s	-CH ₂ -
7.24	1	m	Hc
7.3-7.5	8	m	Ar-H
9.96	1	s	-CHO

$$J_{bc} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{cd} = 2 \text{ Hz}$$

diphenyl alkanedioates



diphenyl

decanedioate

(n=4)

bisphenyl

dodecanedioate

(n=6)

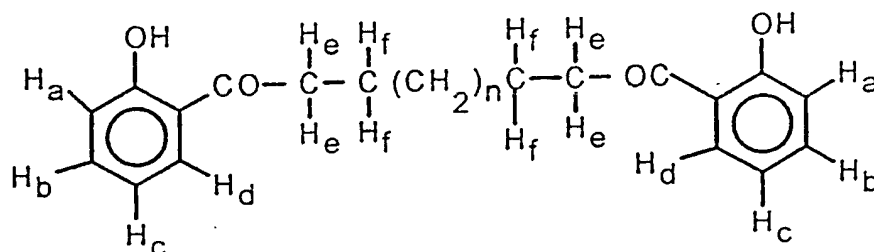
bisphenyl tetra-

decanedioate

(n=8)

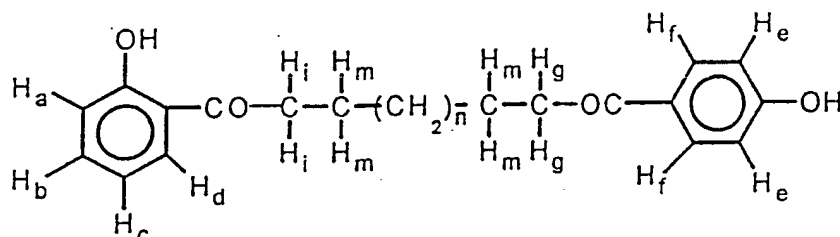
1.33	8	m(b)	-CH ₂ -
1.33	12	m(b)	-CH ₂ -
1.33	16	m(b)	-CH ₂ -
1.75	4	q(b)	He
2.55	4	t	Hd
7.08	4	dd	Ha
7.20	2	tt	Hc
7.36	4	t	Hb

$$J_{ab} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{bc} = 8 \text{ Hz}$$

alpha,omega-bis(2-hydroxyphenyl)alkanediones

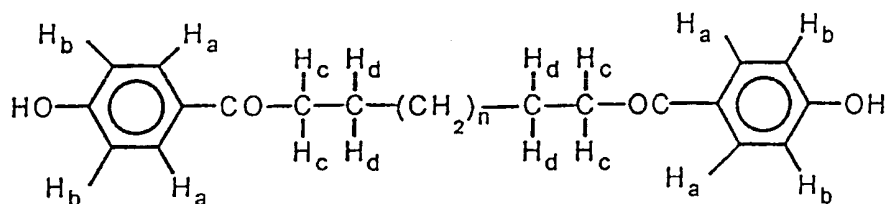
1,10-bis(2-hydroxyphenyl)decane-1,10-dione (n=4)	1.30	8	m(b)	-CH ₂ -
1,12-bis(2-hydroxyphenyl)dodecane-1,12-dione (n=6)	1.30	12	m(b)	-CH ₂ -
1,14-bis(2-hydroxyphenyl)tetradecane-1,14-dione (n=8)	1.30	16	m(b)	-CH ₂ -
	1.75	4	q(b)	Hf
	2.98	4	t	He
	6.9	2	dt	Hc
	6.98	2	dd	Ha
	7.46	2	dt	Hb
	7.76	2	dd	Hd

$$J_{ab} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{bc} = 8 \text{ Hz}, J_{bd} = 2 \text{ Hz}, J_{cd} = 8 \text{ Hz}$$

alpha-(2-hydroxyphenyl)-*omega*-(4-hydroxyphenyl)alkanediones

1-(2-OH-phenyl)-10-(4-OH-phenyl)decane-1,10-dione (n=4)	1.30	8	m(b)	-CH ₂ -
1-(2-OH-phenyl)-12-(4-OH-phenyl)dodecane-1,12-dione (n=6)	1.30	12	m(b)	-CH ₂ -
1-(2-OH-phenyl)-14-(4-OH-phenyl)tetradecane-1,14-dione (n=8)	1.30	16	m(b)	-CH ₂ -
	1.72	4	q(b)	H _m
	2.92	2	t	H _g
	2.97	2	t	H _i
	6.89	1	dt	H _c
	6.92	2	d	H _e
	6.96	1	dd	H _a
	7.45	1	dt	H _b
	7.76	1	dd	H _d
	7.90	2	d	H _f

$J_{ab} = 8 \text{ Hz}$, $J_{ac} = 2 \text{ Hz}$, $J_{bc} = 8 \text{ Hz}$, $J_{bd} = 2 \text{ Hz}$, $J_{cd} = 8 \text{ Hz}$,
 $J_{ef} = 8 \text{ Hz}$

alpha,omega-bis(4-hydroxyphenyl)alkanediones

1,10-bis(4-hydroxy-
phenyl)decane-1,10-
dione (n=4)

1.3

8

m(b)

-CH₂-

1,12-bis(4-hydroxy-
phenyl)dodecane-
1,12-dione (n=6)

1.3

12

m(b)

-CH₂-

1,14-bis(4-hydroxy-
phenyl)tetradecane-
1,14-dione (n=8)

1.3

16

m(b)

-CH₂-

6.92

4

d

Hb

7.9

4

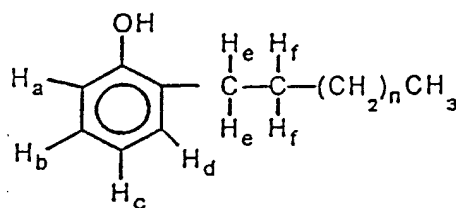
d

Ha

J_{ab} = 8 Hz

^1H -NMR spectra of the alkylphenols and α,ω -bis(hydroxyphenyl)alkanes

2-alkylphenols

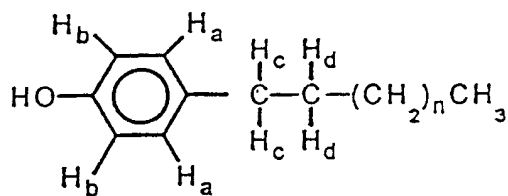


	0.88	3	t(b)	-CH ₃
2-octylphenol (n=5)	1.30	10	m(b)	-CH ₂ -
2-nonylphenol (n=6)	1.30	12	m(b)	-CH ₂ -
2-decylphenol (n=7)	1.30	14	m(b)	-CH ₂ -

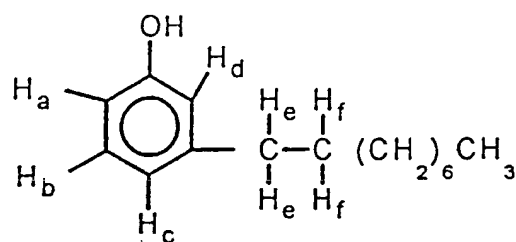
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1.60	2	q(b)	Hf
2.60	2	t	He
6.74	1	dd	Ha
6.86	1	dt	Hc
7.05	1	dd	Hd
7.09	1	dt	Hb

$$J_{ab} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{bc} = 8 \text{ Hz}, J_{bd} = 2 \text{ Hz}, J_{cd} = 8 \text{ Hz}$$

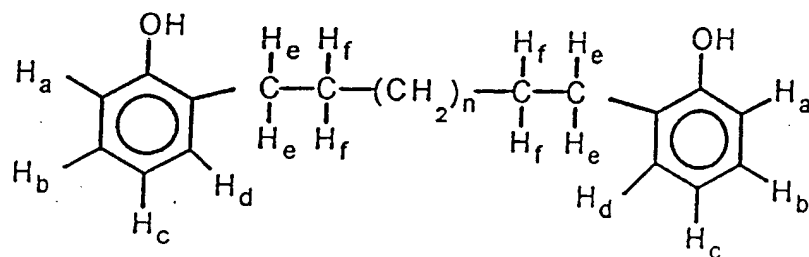
4-alkylphenols

	0.88	3	t(b)	-CH ₃
4-octylphenol (n=5)	1.28	10	m(b)	-CH ₂ -
4-nonylphenol (n=6)	1.28	12	m(b)	-CH ₂ -
4-decylphenol (n=7)	1.28	14	m(b)	-CH ₂ -
	1.56	2	q(b)	Hd
	2.52	2	t	Hc
	6.74	2	d	Hb
	7.02	2	d	Ha
$J_{ab} = 8 \text{ Hz}$				

3-nonylphenol

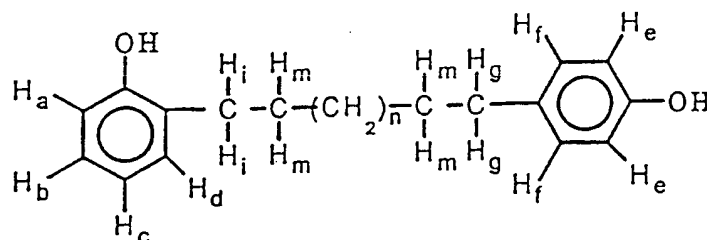
0.88	3	t	-CH ₃
1.27	12	m(b)	-CH ₂ -
1.58	2	q(b)	Hg
2.54	2	t	He
6.64	1	dd	Ha
6.65	1	d	Hd
6.74	1	dd	Hc
7.13	1	dt	Hb

$$J_{ab} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{ad} = 2 \text{ Hz}, J_{bc} = 8 \text{ Hz}, J_{cd} = 2 \text{ Hz}$$

alpha,omega-bis(2-hydroxyphenyl)alkanes

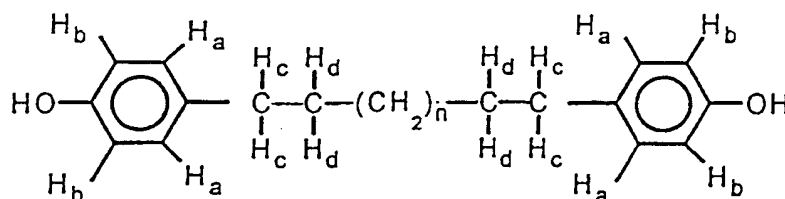
1,10-bis(2-hydroxy phenyl)decane (n=6)	1.28	12	m(b)	-CH ₂ -
1,12-bis(2-hydroxy phenyl)dodecane (n=8)	1.28	16	m(b)	-CH ₂ -
1,14-bis(2-hydroxy phenyl)tetradecane (n=10)	1.28	20	m(b)	-CH ₂ -
	1.60	4	q(b)	Hf
	2.60	4	t	He
	6.75	2	dd	Ha
	6.86	2	dt	Hc
	7.06	2	dd	Hd
	7.10	2	dt	Hb

$$J_{ab} = 8 \text{ Hz}, J_{ac} = 2 \text{ Hz}, J_{bc} = 8 \text{ Hz}, J_{bd} = 2 \text{ Hz}, J_{cd} = 8 \text{ Hz}$$

alpha-(2-hydroxyphenyl)-*omega*-(4-hydroxyphenyl)alkanes

1-(2-OH-ph)-10-(4-OH-ph)decane (n=6)	1.28	12	m(b)	-CH ₂ -
1-(2-OH-ph)-12-(4-OH-ph)dodecane (n=8)	1.28	16	m(b)	-CH ₂ -
1-(2-OH-ph)-14-(4-OH-ph)tetradecane (n=10)	1.28	20	m(b)	-CH ₂ -
	1.60	4	q(b)	Hm
	2.55	2	t	Hg
	2.62	2	t	Hi
	6.73	2	d	He
	6.75	1	dd	Ha
	6.86	1	dt	Hc
	7.02	2	d	Hf
	7.06	1	dd	Hd
	7.10	1	dt	Hb

$J_{ab} = 8 \text{ Hz}$, $J_{ac} = 2 \text{ Hz}$, $J_{bc} = 8 \text{ Hz}$, $J_{bd} = 2 \text{ Hz}$, $J_{cd} = 8 \text{ Hz}$,
 $J_{cf} = 8 \text{ Hz}$

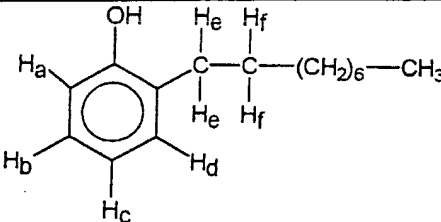
alpha,omega-bis(4-hydroxyphenyl)alkanes

1,10-bis(4-hydroxy-phenyl)decane (n=6)	1.28	12	m(b)	-CH ₂ -
1,12-bis(4-hydroxy-phenyl)dodecane (n=8)	1.28	16	m(b)	-CH ₂ -
1,14-bis(4-hydroxy-phenyl)tetradecane (n=10)	1.28	20	m(b)	-CH ₂ -
	1.60	4	q(b)	Hd
	2.52	4	t	Hc
	6.74	4	d	Hb
	7.02	4	d	Ha

J_{ab} = 8 Hz

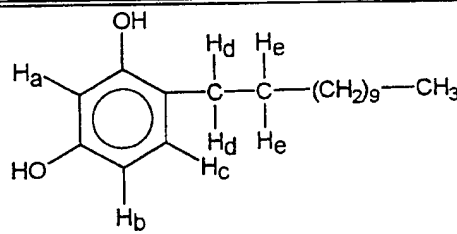
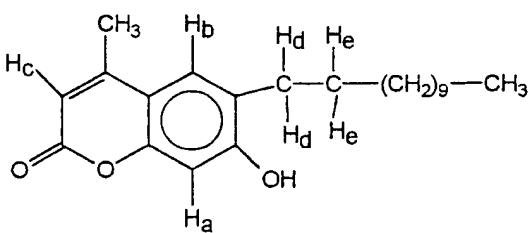
Note: There were only coupling constants of aromatic protons being reported

Table 3 cont

Compounds	δ in ppm	No of protons	Multi- plicity	Assignment
2-nonylphenol derivatives				
				
	0.88	3	t (b)	-CH ₃
	1.30	12	m (b)	-(CH ₂) ₆ -
	1.60	2	m	Hf
	2.24	3	s	Ar-CH ₃
	2.60	2	t	He
Ha=CH ₃	6.77	1	t	Hc
	6.96	2	d	Hb & Hd
J _{bc} /J _{cd} =8Hz				
Hb=CH ₃	6.62	1	d	Ha
	6.72	1	dd	Hc
	7.02	1	d	Hd
J _{ac} =2Hz , J _{cd} =8Hz				
Hc=CH ₃	6.67	1	d	Ha
	6.89	1	dd	Hb
	6.94	1	d	Hd
J _{ab} =8Hz , J _{bd} =2Hz				
Ha=Br	6.72	1	t	Hc
	7.04	1	m	Hd
	7.28	1	dd	Hb

Compounds	δ in ppm	No of protons	Multi- plicity	Assignment
$J_{bc}=8\text{Hz}$, $J_{bd}=2\text{Hz}$, $J_{cd}=8\text{Hz}$, $J_{de}=1\text{Hz}$				
Hc=Br	6.63	1	d	Ha
	7.15	1	dd	Hb
	7.22	1	d	Hd
$J_{ab}=8\text{Hz}$, $J_{bd}=2\text{Hz}$				
Ha=Hc=Br	7.17	1	m	Hd
	7.42	1	d	Hb
$J_{bd}=2\text{Hz}$, $J_{de}=1\text{Hz}$				
Ha=NO ₂	6.89	1	dd	Hc
	7.43	1	m	Hd
	7.95	1	dd	Hb
$J_{bc}=8\text{Hz}$, $J_{bd}=2\text{Hz}$, $J_{cd}=8\text{Hz}$, $J_{de}=1\text{Hz}$				
Hc=NO ₂	6.84	1	d	Ha
	8.00	1	dd	Hb
	8.06	1	d	Hd
$J_{ab}=8\text{Hz}$, $J_{bd}=2\text{Hz}$				
Ha=Br Hc=NO ₂	8.01	1	d	Hd
	8.27	1	d	Hb
Ha=NO ₂ Hc=Br	8.02	1	d	Hd
	8.27	1	d	Hb
$J_{bd}=2\text{Hz}$				
Hb=OH 4-nonyl- resorcinol	6.31	1	d	Ha
	6.35	1	dd	Hc
	6.94	1	d	Hd

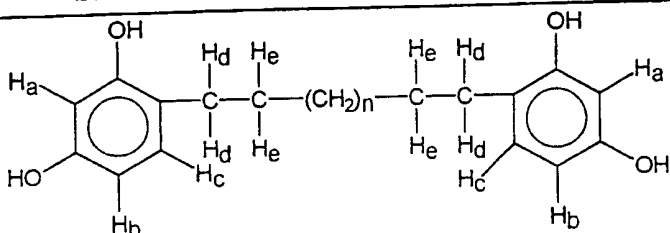
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Compounds	δ in ppm	No of protons	Multi-plicity	Assignment
$J_{ac}=2\text{Hz}$, $J_{cd}=8\text{Hz}$				
Resorcinol/coumarin derivatives				
				
	0.88	3	t (b)	-CH ₃
	1.26	18	m (b)	-(CH ₂) ₉ -
	1.56	2	m	He
	2.58	2	t	Hd
Ha=Br	6.54	1	d	Hb
	6.95	1	d	Hc
$J_{bc}=8\text{Hz}$				
Hb=Br	6.49	1	s	Ha
	7.15	1	s	Hc
Ha=Hb=Br	7.18	1	s	Hd
coumarin derivative				
				
	2.42	3	s	-CH ₃ attached to double bond
	6.12	1	s	Ha
	7.16	1	s	Hc

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Compounds	δ in ppm	No of protons	Multi-plicity	Assignment
	7.30	1	s	Hb
n=4	1.32	8	m(b)	$-(CH_2)_4-$
n=5	1.32	10	m(b)	$-(CH_2)_5-$
	1.60	4	m(b)	Hf
	2.60	4	t	He
	6.75	2	dd	Ha
	6.86	2	td	Hc
	7.07	2	dd	Hd
	7.10	2	td	Hb
$J_{ab} = 8\text{Hz} = J_{bc} = J_{cd}$, $J_{ac} = J_{bd} = 2\text{Hz}$				
n=6	1.32	12	m(b)	$-(CH_2)_6-$
	1.60	4	m(b)	Hf
	2.30	6	s	Ar-CH ₃
	2.60	4	t	He
5 Ha= CH ₃	6.88	2	t	Hc
	7.07	4	d	Hb & Hd
$J_{bc}/J_{cd} = 8\text{Hz}$				
Hb= CH ₃	6.60	2	d	Ha
	6.70	2	dd	Hc
	7.01	2	d	Hd
$J_{ac} = 2\text{Hz}$, $J_{cd} = 8\text{Hz}$				

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	δ in ppm	No of protons	Multi-plicity	Assignment
Hc= CH ₃ Compounds	6.63	2	d	Ha
	6.86	2	dd	Hb
	6.91	2	d	Hd
$J_{ab} = 8\text{Hz}$, $J_{bd} = 2\text{Hz}$				
				
n=4	1.23	8	m(b)	- (CH ₂) ₄ -
n=6	1.23	12	m(b)	- (CH ₂) ₆ -
n=7	1.23	14	m(b)	- (CH ₂) ₇ -
n=8	1.23	16	m(b)	- (CH ₂) ₈ -
	1.53	4	m	He
	2.26	4	t	Hd
	6.05	2	dd	Hb
	6.17	2	d	Ha
	6.63	2	d	Hc
$J_{ab} = 2\text{Hz}$, $J_{bc} = 8\text{Hz}$				
n=6 Ha=CH ₃	¹ H signals from methylene chain are similar to compound No 25 above			
	2.14	6	s	Ar-CH ₃
	6.38	2	d	Hb
	6.75	2	d	Hc
$J_{bc} = 8\text{Hz}$				

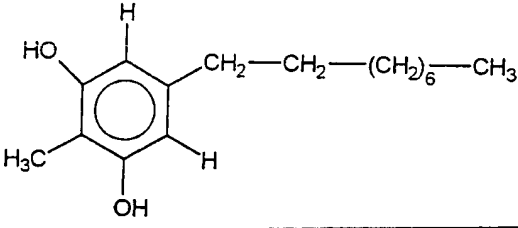
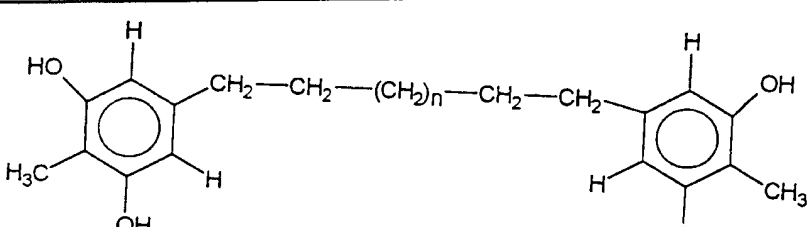
5

10

Compounds	δ in ppm	No of protons	Multi-plicity	Assignment
^1H signals from methylene chain are similar to 25 above				
$R_1=\text{OH}$	6.64	4	m	Ha & Hb
$R_2=\text{Hb}$	6.75	2	dd	Hd
	7.13	2	t	Hc
$J_{ab} = J_{bd} = 2\text{Hz}$, $J_{bc} = J_{cd} = 8\text{Hz}$				
$R_1=\text{OH}$	2.20	6	s	Ar-CH ₃
$R_2=\text{CH}_3$	6.60	2	d	Ha
	6.67	2	dd	Hd
	7.01	2	d	Hc
$R_1=\text{CH}_3$	2.21	6	s	Ar-CH ₃
$R_2=\text{OH}$	6.67	2	d	Hc
	6.87	2	dd	Hd
	6.92	2	d	Ha
$J_{cd} = 8\text{Hz}$, $J_{ad} = 2\text{Hz}$				

Compounds	δ in ppm	No of protons	Multi- plicity	Assignment
1,1-bis-(2-hydroxy-phenyl)-decane	0.86	3	t(b)	-CH ₃
	1.22	14	m(b)	-(CH ₂) ₇ -
	2.12	2	m	-CH ₂ -
	4.47	1	t	= (CH) -
	6.79	2	dd	Ha
	6.90	2	td	Hc
	7.03	2	td	Hb
	7.30	2	dd	Hd
$J_{ab} = J_{bc} = J_{cd} = 8\text{Hz} , J_{ac} = 2\text{Hz} = J_{bd}$				
1,10-bis(2-hydroxy-1-naphthyl)decane	1.30	8	m(b)	-(CH ₂) ₄ -
	1.46	4	m(b)	Hj
	1.65	4	m(b)	Hi
	3.01	4	t	Hg
	7.04	2	d	Ha
	7.31	2	td	Hd
	7.47	2	td	He
	7.61	2	d	Hb
	7.75	2	dd	Hf
	7.91	2	dd	Hc

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Compounds	δ in ppm	No of protons	Multi-plicity	Assignment
$J_{ab} = 8\text{Hz} = J_{cd} = J_{de} = J_{ef}, J_{ce} = J_{df} = 2\text{Hz}$				
				
5 3,5-dihydroxy-4-methyl-phenyl-nonane	0.88	3	t (b)	-CH ₃
	1.26	12	s (b)	-(CH ₂) ₆ -
	1.55	2	m	-CH ₂ -
	2.01	3	s	Ar-CH ₃
	2.45	2	t	Ar-CH ₂ -
	6.24	2	s	Ar-H
				
n=4	1.29	8	m (b)	-(CH ₂) ₄ -
n=6	1.29	12	m (b)	-(CH ₂) ₆ -
n=8	1.29	16	m (b)	-(CH ₂) ₈ -
n=10	1.29	20	m (b)	-(CH ₂) ₁₀ -
10	1.53	4	m	-CH ₂ -
	2.04	6	s	Ar-CH ₃
	2.40	4	t	Ar-CH ₂ -
	6.24	4	s	Ar-H

Note: there are only coupling constants of aromatic proton.

* Summary of ¹³C-NMR (in δ ppm) spectra

2-nonylphenol derivatives

- ^{13}C -NMR of n-C₉H₁₉ chain. 14.19 (1C); 22.75 (1C); 29.41 (1C); 29.55 (1C); 29.58 (1C); 29.64 (2C); 30.86 (1C); 31.97 (1C);
- 5 Aromatic ^{13}C -NMR of substituted phenols:
- 2-bromo-6-nonylphenol. 110.52 (1C); 121.30 (1C); 129.34 (1C); 129.63 (1C); 130.75 (1C); 150.1 (1C).
- .4-bromo-2-nonylphenol. 112.71 (1C); 116.83 (1C); 129.59 (1C); 131.07 (1C); 132.68 (1C); 150.09 (1C).
- 10 2,4-dibromo-6-nonylphenol. 110. (1C); 125.08 (1C); 125.52 (1C); 129.62 (1C); 132.1 (1C); 150.2 (1C).
- 2-nitro-6-nonylphenol. 119.31 (1C); 122.6 (1C); 132.37 (1C); 134.04 (1C); 137.41 (1C); 153.36 (1C).
- 4-nitro-2-nonylphenol. 115.19 (1C); 119.3 (1C); 123.54 (1C); 126.02 (1C); 130.73 (1C); 159.47 (1C).
- 15 4-bromo-2-nitro-6-nonylphenol. 109.92 (1C); 125.07 (1C); 125.51 (1C); 131.18 (1C); 141.26 (1C); 155.58 (1C).
- 4-dodecylresorcinol derivatives
- ^{13}C -NMR of n-C₁₂H₂₅ chain. 14.13 (1C); 22.7 (1C); 29.37 (1C); 29.41 (1C); 29.53 (1C); 29.62 (1C); 29.65 (1C); 29.68 (2C); 29.85 (1C); 30.17 (1C); 31.93 (1C).
- Aromatic ^{13}C -NMR of substituted resorcinols:
- 2-bromo-6-dodecylresorcinol. 99.63 (1C); 107.37 (1C); 122 (1C); 129.38 (1C); 150.24 (1C); 150.61 (1C).
- 25 4-bromo-6-dodecylresorcinol. 100.52 (1C); 103.17 (1C); 123 (1C); 132.17 (1C); 150.79 (1C); 154 (1C).
- 2,4-dibromo-6-dodecylresorcinol. 98.68 (1C); 99.62 (1C); 123.46 (1C); 131.1 (1C); 147.3 (1C); 150.52 (1C)
- 6-dodecyl-7-hydroxy-4-methylcoumarin
- 30 (^{13}C -NMR of n-C₁₂H₂₅ are similar to those of resorcinol above)
- Aromatic ^{13}C . 102.62 (1C); 110.7 (1C); 112.37 (1C); 125 (1C); 127.28 (1C); 153.35 (1C); 159.12 (1C); 162.3 (1C).
- 1,11-bis(2,4-dihydroxyphenyl)undecane: 29.37 (2C); 29.41 (2C); 29.43 (3C); 29.46 (2C); 30.13 (2C); 102.93 (2C); 106.68 (2C); 120.50 (2C); 130.29 (2C); 155.24 (2C); 155.57 (2C).
- 35 1,12-bis(2,4-dihydroxyphenyl)dodecane: 28.91 (2C); 29.02

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- (2C); 29.05 (4C); 29.09 (2C); 29.69 (2C); 102.39 (2C);
105.79 (2C); 119.68 (2C); 129.54 (2C); 155.11 (2C);
155.45 (2C).
- 5 1,10-bis(2,4-dihydroxy-3-methylphenyl)decane: 8.51 (2C);
29.43 (2C); 29.47 (2C); 29.49 (2C); 29.86 (2C); 30.06
(2C); 107 (2C); 110.42 (2C); 119.76 (2C); 126.6 (2C);
152.65 (2C); 153.55 (2C).
- 10 1,10-bis(3-hydroxy-4-methylphenyl)decane: 15.31 (2C);
29.25 (2C); 29.46 (2C); 29.5 (2C); 31.41 (2C); 35.47
(2C); 114.92 (2C); 120.59 (2C); 120.76 (2C); 130.72 (2C);
142.31 (2C); 153.52 (2C).
- 15 2-methyl-5-nonylresorcinol: 7.73 (1C); 14.12 (1C); 22.69
(1C); 29.3 (1C); 29.35 (1C); 29.54 (1C); 31.21 (1C);
31.91 (1C); 107.31 (1C); 107.8 (2C); 142.05 (1C); 154.46
(2C).
- 20 1,8-bis(3,5-dihydroxy-4-methylphenyl)octane: 8.37 (2C);
29.93 (2C); 30.18 (2C); 32.11 (2C); 36.24 (2C); 107.34
(2C); 107.47 (4C); 141.59 (2C); 156.89 (4C).
- 1,10-bis(3,5-dihydroxy-4-methylphenyl)decane: 8.34 (2C);
29.94 (2C); 30.32 (4C); 32.13 (2C); 36.25 (2C); 107.34
(2C); 107.45 (4C); 141.59 (2C); 156.9 (4C).
- 25 1,12-bis(3,5-dihydroxy-4-methylphenyl)dodecane: 8.37
(2C); 29.97 (2C); 30.26 (2C); 30.34 (2C); 30.38 (2C);
32.15 (2C); 36.27 (2C); 107.34 (2C); 107.45 (4C); 141.58
(2C); 156.9 (4C).

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Table 4: Chemical ionisation mass spectra of the synthesised alkylphenols and α, ω -bis (hydroxyphenyl)alkanes.

Mass spectra of the intermediate products

m/e	intensity(%)	inference
2-octanoylphenol		
261	4	M + 41
249	11	M + 29
221	100	M + 1
127	6	loss of phenol
2-nonanoylphenol		
275	5	M + 41
263	10	M + 29
235	100	M + 1
141	9	loss of phenol
2-decanoylphenol		
289	5	M + 41
277	16	M + 29
249	100	M + 1
155	15	loss of phenol
121	16	2-OH-benzoyl cation
4-octanoylphenol		
221	100	M + 1
127	13	loss of phenol
113	6	loss of octane

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4-nonanoylphenol

235	100	M + 1
221	5	loss of CH ₃ group
141	6	loss of phenol
135	7	loss of heptane

4-decanoylphenol

289	5	M + 41
277	12	M + 29
249	100	M + 1
221	8	loss of ethylene
155	10	loss of phenol
135	8	loss of octane

3-benzyloxybenzaldehyde

253	5	M + 41
241	12	M + 29
213	100	M + 1
135	16	loss of benzene

diphenyl decanedioate

383	7	M + 29
355	12	M + 1
261	100	loss of phenol

diphenyl dodecanedioate

411	20	M + 29
383	5	M + 1
289	100	loss of phenol

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diphenyl tetradecanedioate .

439	15	M + 29
411	4	M + 1
317	100	loss of phenol

1,10-bis(2-hydroxyphenyl)decane-1,10-dione

383	6	M + 29
355	100	M + 1
261	10	loss of phenol

1,12-bis(2-hydroxyphenyl)dodecane-1,12-dione

423	8	M + 41
411	20	M + 29
383	100	M + 1
289	5	loss of phenol

1,14-bis(2-hydroxyphenyl)tetradecane-1,14-dione

451	6	M + 41
439	16	M + 29
411	100	M + 1
317	5	loss of phenol

1-(2-hydroxyphenyl)-10-(4-hydroxyphenyl)decane-1,10-dione

383	8	M + 29
355	100	M + 1
327	10	loss of 28 mass unit
261	14	loss of phenol
135	25	$C_6H_4(OH)COCH_2^+$ ion
121	15	$C_6H_4(OH)CO^+$ ion

1-(2-hydroxyphenyl)-12-(4-hydroxyphenyl)dodecane-1,12-dione

423	8	M + 41
411	20	M + 29
383	100	M + 1
289	10	loss of phenol
121	5	$C_6H_4(OH)CO^+$ ion

1-(2-hydroxyphenyl)-14-(4-hydroxyphenyl)tetradecane-1,14-dione

451	6	M + 41
439	18	M + 29
411	100	M + 1
355	18	loss of butene
317	10	loss of phenol

1,10-bis(4-hydroxyphenyl)decane-1,10-dione

395	6	M + 41
383	15	M + 29
355	100	M + 1
261	8	loss of phenol
121	15	$C_6H_4(OH)CO^+$ ion

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1,12-bis(4-hydroxyphenyl)dodecane-1,12-dione

423	6	M + 41
411	16	M + 29
383	100	M + 1
289	8	loss of phenol
137	18	$C_6H_4(OH)C(OH)^+CH_3$
123	12	$C_6H_4(OH)C(OH)^+H$

1,14-bis(4-hydroxyphenyl)tetradecane-1,14-dione

451	5	M + 41
439	18	M + 29
411	100	M + 1
317	8	loss of phenol
121	12	4-OH-benzoyl cation

Mass spectra of alkylphenols and α,ω -bis(hydroxyphenyl)alkanes

2- and 4-octylphenols

247	8	M + 41
235	16	M + 29
207	100	M + 1
107	20	$C_6H_4(OH)CH_2^+$ ion

2-, 3- and 4-nonylphenols

261	6	M + 41
249	12	M + 29
221	100	M + 1
107	18	$C_6H_4(OH)CH_2^+$ ion

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2- and 4-decylphenol

275	5	M + 41
263	16	M + 29
235	100	M + 1
107	16	C ₆ H ₄ (OH)CH ₂ ⁺ ion

1,10-bis(hydroxyphenyl)decanes and

1-(2-hydroxyphenyl)-10-(4-hydroxyphenyl)decane

367	6	M + 41
355	20	M + 29
327	100	M + 1
107	15	C ₆ H ₄ (OH)CH ₂ ⁺ ion

1,12-bis(hydroxyphenyl)dodecanes and

1-(hydroxyphenyl)-12-(4-hydroxyphenyl)dodecane

395	5	M + 41
383	20	M + 29
355	100	M + 1
107	6	C ₆ H ₄ (OH)CH ₂ ⁺ ion

1,14-bis(hydroxyphenyl)tetradecanes and

1-(2-hydroxyphenyl)-14-(4-hydroxyphenyl)tetradecane

423	5	M + 41
411	20	M + 29
383	100	M + 1
107	5	C ₆ H ₄ (OH)CH ₂ ⁺ ion

Note: The signals with intensity less than 5% were not accounted.

Table 4 continued

	m/e	INTENSITY(%)	INFERENCE
	2-,3- and 4-methyl-6-nonylphenol		
	275	5	M + 41
5	263	20	M + 29
	235	100	M + 1
	219	5	loss of methane
	121	10 - 20	loss of C ₈ H ₁₈
	2- and 4-bromo-6-nonylphenol		
10	327	10 - 20	M + 29
	299	100	M + 1
	185	15	loss of C ₈ H ₁₈
	127	5 - 10	(C ₉ H ₁₉) ⁺
	2,4-dibromo-6-nonylphenol		
15	419	15	M + 41
	407	25	M + 29
	377	100	M + 1
	363	15	loss of methane
	299	18	loss of Br
20	265	20	loss of octane
	127	45	(C ₉ H ₁₉) ⁺
	2- and 4-nitro-6-nonylphenol		
	306	5	M + 41
	294	15	M + 29
25	266	100	M + 1
	250	5	loss of methane

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	m/e	INTENSITY (%)	INFERENCE
	236	10 - 12	loss of ethane
	2-bromo-4-nitro-6-nonylphenol/4-bromo-2-nitro-6-nonylphenol		
	372	12	M + 29
	344	100	M + 1
5	314	10	loss of ethane
	266	20	loss of Br
	248	10	loss of heptene
	4-nonylresorcinol		
	277	8	M + 41
10	265	20	M + 29
	237	100	M + 1
	123	30	(C ₇ H ₇ O ₂) ⁺
	2- and 4-bromo-6-dodecylresorcinol		
	385	15	M + 29
15	357	100	M + 1
	279	10 - 20	loss of Br
	201	15 - 25	loss of C ₁₁ H ₂₄
	135	5 - 10	(C ₈ H ₇ O ₂) ⁺
	2,4-dibromo-6-dodecylresorcinol		
20	477	8	M + 41
	465	15	M + 29
	437	100	M + 1
	357	45	loss of Br
	281	20	loss of C ₁₁ H ₂₄
25	201	10	loss of 236 mass unit

	m/e	INTENSITY (%)	INFERENCE
	135	15	$(C_8H_7O_2)^+$
	6-dodecyl-7-hydroxy-4-methylcoumarin		
	385	8	M + 41
	373	15	M + 29
5	345	100	M + 1
	135	10	$(C_8H_7O_2)^+$
	1,8-bis(2-hydroxyphenyl)octane		
	339	5	M + 41
	327	20	M + 29
10	299	100	M + 1
	135	30	$(C_9H_{11}O)^+$
	1,9-bis(2-hydroxyphenyl)nonane		
	353	6	M + 41
	341	20	M + 29
15	313	100	M + 1
	1,10-bis[2-hydroxy-3(4-and 5-)methylphenyl]decanes		
	395	5	M + 41
	383	20 - 25	M + 29
	355	100	M + 1
20	1,8-bis(2,4-dihydroxyphenyl)octane		
	371	5	M + 41
	359	25	M + 29
	331	100	M + 1
	315	5	loss of methane
25	1,10-bis(2,4-dihydroxyphenyl)decane		

	m/e	INTENSITY (%)	INFERENCE
	399	5	M + 41
	387	20	M + 29
	359	100	M + 1
	1,11-bis(2,4-dihydroxyphenyl)undecane		
5	413	10	M + 41
	401	30	M + 29
	373	100	M + 1
	123	5	(C ₇ H ₇ O ₂) ⁺
	1,12-bis(2,4-dihydroxyphenyl)dodecane		
10	427	5	M + 41
	415	30	M + 29
	387	100	M + 1
	1,10-bis(2,4-dihydroxy-3-methylphenyl)decane		
	427	5	M + 41
15	415	25	M + 29
	401	15	M + 15
	387	100	M + 1
	1,10-bis(3-hydroxyphenyl)decane		
	367	8	M + 41
20	355	25	M + 29
	327	100	M + 1
	1,10-bis[3-hydroxy-4-methyl (and 4-hydroxy-3-methyl)phenyl]decanes		
	395	5	M + 41
25	383	20	M + 29

	m/e	INTENSITY (%)	INFERENCE
	355	100	M + 1
	1,1-bis(2-hydroxyphenyl)decane		
	327	30	M + 1
	233	100	loss of phenol
5	135	20	(C ₉ H ₁₁ O) ⁺
	107	5	(C ₇ H ₇ O) ⁺
	1,10-bis(2-hydroxy-1-naphthyl)decane		
	467	5	M + 41
	455	15	M + 29
10	427	70	M + 1
	135	50	(C ₉ H ₁₁ O) ⁺
	119	100	
	2-methyl-5-nonylresorcinol		
	291	5	M + 41
15	279	20	M + 29
	251	100	M + 1
	235	8	loss of methane
	135	8	(C ₈ H ₇ O ₂) ⁺
	1,8-bis(3,5-dihydroxy-4-methylphenyl)octane		
20	399	8	M + 41
	387	25	M + 29
	359	100	M + 1
	135	5	(C ₈ H ₇ O ₂) ⁺
	1,10-bis(3,5-dihydroxy-4-methylphenyl)decane		
25	427	5	M + 41

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	m/e	INTENSITY (%)	INFERENCE
	415	20	M + 29
	387	100	M + 1
	135	5	(C ₈ H ₇ O ₂) ⁺
	1,12-bis(3,5-dihydroxy-4-methylphenyl)dodecane		
5	455	5	M + 41
	443	25	M + 29
	415	100	M + 1
	1,14-bis(3,5-dihydroxy-4-methylphenyl)tetradecane (striatol)		
	483	5	M + 41
10	471	20	M + 29
	443	100	M + 1
	135	5	(C ₈ H ₇ O ₂) ⁺

Note: signals with intensity less than 5% were not accounted.

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EXPERIMENTAL - NATURAL PRODUCTS

NOVEL RESORCINOLS FROM GREVILLEA ROBUSTA AND THEIR INHIBITORY ACTIVITY TO ERYTHROCYTE Ca^{2+} -ATPase

General experimental procedures for extraction and isolation - The mobile phase was MeOH/H₂O (82:18). Flow rate was 1.0 ml/min; Preparative HPLC were carried out with a Altex 100 solvent delivery system, equipped with Altex UV detector at 254nm, a Activon Partisil ODS-3 column, 9x500mm was used. The mobile phase was a gradient of MeOH/H₂O (3:7) to MeOH in 60 min. or an isocratic mobile phase 65% CH₃CN in H₂O, flow rate was 2.5 ml/min. 6251, 6252 were separated by these systems.

Plant Material - The stem of *Grevillea robusta* was collected in Sydney, Australia. A voucher specimen is available for inspection at the Department of Pharmacy, The University of Sydney. The stem wood, 5-10 cm in diameter, was sliced by an electrical plane and air dried.

Extraction and Isolation - A sample of 2 kg was extracted by percolation with CHCl₃/EtOH (1:1) for 3 days twice. After concentration of the extract in vacuo, the residue (20g) was chromatographed by Silica gel short column vacuum chromatography collecting 250ml fractions. The Ca^{2+} -ATPase assay gave an inhibitory activity of 50% at a concentration of 0.5mg/ml. Fractions 1 to 4 (3.82g) were eluted with petroleum/EtOAc (9:1- 2:1). Fractions 7-9 (11.91g), eluted from CHCl₃/MeOH(2:1), were inactive in the assay. They were not further studied. Fraction 5 (2.78g), 6 (0.13g), 6b(1.38g) were eluted with CHCl₃/EtOAc(2:1), CHCl₃/MeOH (9:1), (8:2) respectively and gave strong inhibitory activity. Fraction 5 was further short column vacuum chromatographed with CHCl₃/EtOH (95:5) and gave grevillol (0.74g). Fraction 6b was similarly chromatographed with CH₂Cl₂/ EtOAc (4:1) to give 6b2 (37.4mg). Fraction 6 was similarly separated into 10 fractions (each 50ml) with petroleum /EtOAc (2:1), CH₂Cl₂/EtOAc (2:1), (1:1), (1:2), EtOAc, EtOAc/CH₃CN (1:1). Only fraction 4-5 (63.4mg) from CH₂Cl₂/EtOAc (2:1)

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gave strong inhibitory activity, 74% inhibition at 0.04mg/ml. This fraction was then separated by gradient HPLC to give synaptic aldehyde (623, 1.8mg), and a nonpolar fraction 625 which was finally separated into
5 6251 (6.6mg) and 6252 (1.3mg) by preparative TLC, with $\text{CHCl}_3/\text{EtOH}$ (95:5) as a solvent.

The methylation and ozonolysis is a method from Barrow R A, Capon R J (1991) Alkyl and alkenyl resorcinols from an Australian marine sponge, *Haliclona*
10 sp. Aust.J.Chem 44 1393-1409. The sample to be methylated (2-8 mg) was stirred in acetone (3 ml) with K_2CO_3 (200mg) and CH_3I (0.5ml) at room temperature for 20 h. The methylated products were isolated by preparative TLC with $\text{CHCl}_3/\text{EtOH}$ (9.3:0.7) for 6b2, petroleum / EtOAc
15 (8:2) for 6251, 6252. The methylated compounds (0.5-1 mg) in CS_2 (2ml) at -78 C were ozonolysed with a stream of O_3 and then triphenylphosphine (2mg) added. The reaction mixtures were analysed directly by CI-MS.

The long chain resorcinols gave a characteristic
20 purple colour on TLC after exposure to I_2 vapour and being left on the bench overnight, while resorcinol, resorcinylic acid, orcinol gave a dust brown colour.

Grebustol-A (6251)- Colourless oil; R_f 0.51 ($\text{CHCl}_3/\text{EtOH}$ 9:1); $^1\text{H-NMR}$ (CDCl_3 , ppm) 1.26-1.35 (m, 12H, CH_2), 1.55 (m, 4H, ArCH_2CH_2), 2.00 (m, 4H, $\text{CH}=\text{CHCH}_2$), 2.10 (s, 3H, ArCH_3), 2.42-2.50 (m, 4H, ArCH_2), 4.70 (br.s., OH), 5.32-5.39 (m, 2H, $\text{CH}=\text{CH}$), 6.17(t, $J=2.0\text{Hz}$, 1H, ArH), 6.24(br.s., 4H, ArH); $^{13}\text{C-NMR}$ 156.5, 154.4, 142.6, 142.0, 130.0, 129.7, 108.1, 107.9, 100.2, 35.9, 35.6, 31.3,
30 31.1, 29.8, 29.7, 29.6, 29.4, 29.2, 29.1, 27.2, 7.9; CI-MS (CH_4) m/z 427{M+1}, 397, 285, 257, 229. 207; UV max(MeOH) 208.4 ($\log\epsilon$ 4.48), 274.4 (3.35), 279.4 (3.33).

Methylated Grebustol-A (6251m)- $^1\text{H-NMR}$ 1.26-1.38 (m, 12H, CH_2), 1.55 (m, 4H, ArCH_2CH_2), 2.00 (m, 4H, $=\text{CHCH}_2$),
35 2.06 (s, 3H, ArCH_3), 2.55 (m, 4H, ArCH_2), 3.78 (s, 6H, 2 X OCH_3), 3.81 (s, 6H, 2 X OCH_3), 5.32-5.41 (m, 2H, $\text{CH}=\text{CH}$), 6.29 (t, $J=2.3\text{Hz}$, 1H, 2-H), 6.34 (d, $J=2.3\text{Hz}$, 2H, 4, 6, -H), 6.36 (s, 2H, 4'6'-H); CI-MS {M+1}⁺ 483, EI-MS 482{M}⁺

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(32), 410(6), 386(13), 368(8), 353(3), 341(8), 149(12), 109(9); Ozonolysis of 6251m, $^1\text{H-NMR}$ 3.81, 3.78, 9.76, 9.77 (CHO), CI-MS 279, 237.

Norstriatol-B (6252)- colourless oil, R_f 0.55
5 (CHCl₃/EtOH 9:1); $^1\text{H-NMR}$ (CDCl₃, ppm) 1.25 (m, 12H, CH₂), 1.58 (m, 4H, CH₂), 1.91 (m, 4H, =CH CH₂), 2.26 (m, 2H, 1-CH₂), 2.60 (m, 2H, 14-CH₂), 5.30 (m, 2H, CH=CH), 4.66 (br.s., OH), 6.45-6.49 (m, 4H, ArH); CI-MS 411{M+1}⁺, 317, 285, 257; UV max (MeOH) 206.4 (log ϵ 4.61), 279.2
10 (3.49).

Methylated norstriatol-B (6252m)- $^1\text{H-NMR}$ 1.25 (m, 12H, CH₂), 1.54 (m, 4H, CH₂), 1.87 (m, 4H, CH=CHCH₂), 2.23 (m, 2H, 1-CH₂), 2.66 (m, 2H, 14-CH₂), 3.68 (d, J=3.0Hz, 3H, 22-OCH₃), 3.69 (d, J=1.40 Hz, 6H, 17,19-OCH₃), 3.83
15 (d, J=1.45Hz, 3H, 24-OCH₃), 5.30 (m, 2H, CH=CH), 6.42-6.45 (m, 4H, ArH); CI-MS 467 {m+1}⁺; EI-MS 466 {m}⁺ (100), 451(2), 302(5), 149(10);

$^{13}\text{C-NMR}$, 56.0, 56.1, 96.5, 104.8, 105.0;

Methylated striatol-B- $^{13}\text{C-NMR}$ 56.0 , 56.1, 96.5,
20 105.0, 130.3, 145.5.

Results and Discussion

Grebustol-A (6251) - Compound (II)

Grebustol-A (6251) (6.6 mg, 3.3ppm), molecular weight 426 (CI-MS), UV Max 274nm, is different from
25 striatol in having a double bond in the alkyl chain and a single benzyl methyl group as indicated by the $^1\text{H-NMR}$ signals at 5.32-5.39 ppm (m, 2H), and 2.10 ppm (s, 3H). The mass spectrum and $^1\text{H-NMR}$ spectrum of the methylated product revealed the presence of four OCH₃ groups (EI-MS
30 482), $^1\text{H-NMR}$ 3.78 ppm (s, 6H) 3.81 (s, 6H)), $^1\text{H-NMR}$ H-H COSY found the coupling of the signals 1.6-1.55, 1.26-2.00, 1.55-2.42, 2.00-5.35 ppm, but no coupling between the signals 1.55 and 2.00 ppm. This excludes the possibility of the double bond at C-10,11. $^1\text{H-NMR}$ data
35 indicated a non-symmetric structure. The CI-MS of ozonolysis products indicated aldehydes of molecular weight 278 and 236, consistent with a C-8,9 (m=7, n=5), or , C-9,10 (m=8, n=4) position for the double bond.

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Norstriatol-B (6252) - Compound (III)

Norstriatol-B (6252) (1.3mg, 0.65ppm) is a desmethyl product of striatol-B. The CI-MS (CH₄ reagent gas) revealed a molecular weight 410. UV Max 279nm. The
5 ¹H-NMR , UV, mass spectrum of norstriatol-B was consistent with the reported data for striatol-B [Ridley D D, et al (1970) Chemical studies of the Proteaceae IV Aust.J.Chem. 23, 147-183] and an authentic sample of
10 striatol-B. It is a biphenyl derivative rather than a diphenyl ether derivative as with robustol [Cannon J R, et al (1973) Phenolic constituents of *Grevillea robusta* (Proteaceae). The structure of robustol, a novel macrocyclic phenol Aust.J.Chem. 26, 2257-2275]. The
15 methylation of norstriatol-B gave tetramethyl norstriatol-B, which was identical to the substance formed by methylation of authentic striatol-B.

Table 1 shows that the inhibitory effect of grevillol to Ca²⁺-ATPase was weak. The most potent
20 compounds are striatol and grebustol-A, with IC₅₀ of 16 and 17μM respectively. Without the benzylic methyl group, the activity was weaker, as in grebustol-B. Bisorstriatol gave only 69.1% inhibition at the
25 concentration of 500 μM. After the methylation of the phenolic hydroxy group, the activity of grebustol-B was lost. The inhibitory activity of striatol has been confirmed with purified erythrocyte Ca²⁺-ATPase.

These results indicate the phenolic hydroxy groups were necessary for the inhibition to occur. The benzylic
30 methyl group between the hydroxy groups enhances the activity. Also the double bond in the alkyl chain increased the inhibitory activity to Ca²⁺-ATPase.

Erythrocyte membranes

Calmodulin-depleted erythrocyte membranes were prepared by continuous filtration through a hollow fibre,
35 Ashahi plasma separator as described by W S Price, B D Roufogalis, P W Kuchel (1989), A simple and inexpensive method for preparing erythrocyte membranes by filtration through a hollow-filter system, Anal. Biochem., 179, 190-

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193. Packed cells were obtained from the New South Wales Red Cross Transfusion Service, Sydney. The whole preparation was carried out at 4°C. 1 unit of packed red cells was washed 3 times with isotonic buffer, containing
5 130 mM KCl, 20 mM Tris-HCl (pH 7.4), and the cells were collected by centrifugation at 4,000 RPM. The cells were haemolysed by the buffer containing 1 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.5 mM PMSF (phenylmethyl sulfonyl fluoride). The haemolyzate mixture was passed through
10 the hollow fibre system until the membrane appeared white, and then washed with 10 mM potassium Hepes (pH 7.4). The membranes were collected by centrifugation at 10,000 RPM for 20 min, the membranes were resuspended in storage buffer containing 130 mM KCl, 2 mM
15 dithiothreitol, 0.5 mM MgCl₂, 20 mM potassium-Hepes (pH 7.5). The membranes with protein concentration of 1.5-5.4 mg/ml were stored at -80°C until required.

Ca²⁺-ATPase assay

Erythrocyte membranes (0.071-0.098 mg/ml) were
20 incubated at 37°C for one hour in a total volume of 0.4 ml of 65 mM KCl, 20 mM potassium-Hepes, 5 mM MgCl₂, 50 µM CaCl₂, 0.1 µM calmodulin, 0.1 mM EGTA. The reaction was started by adding 2 mM ATP (pH 7.4). The phosphate liberated to the medium was determined spectrometrically
25 according to the procedure of B U Raess, F F Vincenzi (1980), A semi-automated method for the determination of multiple membrane ATPase activities, J. Pharmacological Methods 4, 273-283. The activity of the Mg²⁺-ATPase (assayed in the absence of added CaCl₂) was subtracted
30 from the total activity assayed in the presence of Ca²⁺. Phenols were dissolved in dimethyl sulfoxide (DMSO), the final concentration of DMSO in the assay mixture was 2.5%. DMSO itself had no effect on ATPase activities. A concentrated solution of the test substances was added to
35 the reaction medium before adding ATP. Protein concentration was determined according to the method of O H Lowry, N J Rosebrough, A L Farr, R J Randall (1951) J. Biol. Chem. 193, 265-275; bovine serum albumin was used

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as a standard. The concentration of the free Ca^{2+} were calculated by computer using a program of D A Goldstein (1979) Calculation of the concentrations of free cations and cation-ligand complexes in solutions containing multiple divalent cations and ligands, Biophys.J., 26, 235-242.

Control specific activity of the ATPases were (unit: nmoles/mg protein/min): Ca^{2+} -ATPase, was 25.8 ± 4.0 (n=20), calmodulin stimulated Ca^{2+} -ATPase was 63.5 ± 7.9 (n=16), while the Mg^{2+} -ATPase was 7.4 ± 1.0 (n=20). The enzyme was inhibited by NAP-taurine by 60% at a concentration of 25 μM as shown by A Minocherhomjee, B D Roufogalis (1982), Selective antagonism of the Ca transport ATPase of the red cell membrane by N-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate (NAP-taurine), J.Biol.Chem. 257, 5426-5430.

Ca^{2+} -ATPase assay (microplate method)

Erythrocyte membranes were incubated at 37°C for one hour in a total volume of 60 μl of 65 mM KCl, 50 mM HEPES (pH=7.4), 5 mM MgCl_2 , 50 μM CaCl_2 , 0.1 mM EGTA and in absence and presence of calmodulin (0.3 μM). Test compounds were dissolved in dimethyl sulfoxide DMSO and 2 μl was added to the assay mixture prior the addition of ATP. The final concentration of DMSO in the assay mixture was 3%. The reaction was started by adding 2 mM ATP (pH=7.4). After 1 hour of incubation, colouring agent (180 μl) was added and incubated at 37°C for 1/2hrs. The phosphate liberated to the assay medium was determined spectrometrically using a microplate reader at 750nm. The activity of the Mg^{2+} -ATPase (assayed in the absence of added CaCl_2) was subtracted from the total activity assayed in the presence of Ca^{2+} . The results are shown in Table 1A.

ABBREVIATIONS

ATP	:	Adenosine triphosphate
ATPase	:	Adenosine triphosphatase
BHT	:	2,6-di-tert-butyl-4-methylphenol
CI-MS	:	Chemical ionisation mass spectrometry

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	DMSO	:	Dimethyl sulfoxide
	EI-MS	:	Electron impact mass spectrometry
	EGTA	:	ethylenebis(oxyethylenenitrilo)tetraacetic acid
5	HEPES	:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
	HPLC	:	High-performance liquid chromatography
	LDA	:	Lithium diisopropylamide
	NAP	:	N-(4-azido-2-nitrophenyl)-2-aminoethanesulfonic acid
10			
	NMR	:	Nuclear magnetic resonance
	PMSF	:	Phenylmethyl sulfonyl fluoride
	SDS	:	Sodium dodecylsulfate
	TLC	:	Thin layer chromatography

15 TOXICITY - BRINE SHRIMP ASSAY

The brine shrimp assay procedure determines LC₅₀ values of active compounds. Activities of a broad range of known active compounds are manifested as toxicity to brine shrimp (*Artemia salina* Leach). There are many applications of the assay including analysis of toxic substances, anaesthetics, morphine-like substances and cocarcinogenicity of phorbol esters. The assay shows good correlation with some cytotoxicities and its utility as a prescreen for some antitumour activities has been recently confirmed.

DMSO (dimethyl sulfoxide) was the solvent of choice because of its good solubilising properties and also because the phenolic substances used in the Ca²⁺-ATPase inhibition study were already prepared with DMSO.

The method for testing solvent toxicity used was basically that reported by J L McLaughlin in Methods of Plant Biochemistry (1991), vol. 6 (K Hostettman, ed.), Academ Press, London, 1-32. DMSO solutions of the substances to be tested were added directly to the vials containing the brine shrimp. As the concentration of DMSO that we wished to use was higher than the recommended 1% v/v testing of the toxicity of the DMSO was therefore necessary. The concentrations of DMSO tested on the

- 85 -

shrimp, along with the results from the assay which was done in duplicate are listed in Table 5.

Table 5. Concentrations of DMSO tested.

	Conc (% v/v)	% Deaths
5	0	0
	1	0
	2	0
	3	0
	4	0
10	5	9
	7	12
	9	18
	11	57
	13	96
15	15	100
	20	100
	25	100

No toxicity towards brine shrimp was observed in a 24 hour period for concentrations of DMSO in brine up to 4% v/v.

Bioassay

Brine shrimp toxicity was assayed, except for some minor modifications, according to the method of McLaughlin et al as reported in Brine Shrimp: A convenient general bioassay for active plant constituents, B N Meyer, N R Ferrigni, J E Putman, L B Jacobsen, D E Nholts and J L McLaughlin. Planta Medica (1982), 45, 31-34 and Crown gall tumours on potato discs and brine shrimp lethality: Two simple bioassay for higher plant screening and fractionation. J L McLaughlin. Methods of Plant Biochemistry (1991), vol. 6 (K Hostettman, ed.), Academ Press, London, 1-32. Ten shrimp were added transferred to each of the vials and the volume adjusted to 4.9 mL. Each dose was performed in triplicate, including the control. In quick succession, the appropriate volume of additional DMSO for each dose,

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required to achieve a final concentration of 2%, was added before the appropriate volume of test solution. The vials were gently mixed and the time noted. After 24 hours, the number of survivors were counted and % mortality was determined. The test compounds were assayed at concentrations of 100 μM , 25 μM , 5 μM , 1 μM and 0.2 μM (and where appropriate concentrations of 0.04 μM and 0.008 μM).

The brine shrimp were able to survive without food in the vials over the 24 hour period and were therefore not fed.

The dose-response curves were constructed using the Sigmaplot computer program and the LC_{50} value was calculated from the intersection point of the curve and the 50 % mortality line. The LC_{50} values were expressed in both μM and $\mu\text{g/mL}$.

Table 6. LC_{50} values from brine shrimp bioassay

MWt	Compound	LC_{50}	
		μM	$\mu\text{g/mL}$
206	2-octylphenol	1.5	0.31
220	2-nonylphenol	0.48	0.11
234	2-decylphenol	0.68	0.16
220	3-nonylphenol	0.32	0.070
220	4-nonylphenol	0.40	0.088
234	4-decylphenol	0.27	0.064
234	2-nonanoylphenol	0.63	0.15
234	4-nonanoylphenol	0.10	0.024
176	2-cyclohexylphenol	>25	>4.4
176	4-cyclohexylphenol	>25	>4.4
298	1,8-bis(2-hydroxyphenyl)octane	3	0.89
326	1,10-bis(2-hydroxyphenyl)decane	1.9	0.62

	Mwt	Compound	LC ₅₀	
			μM	$\mu\text{g/mL}$
	326	1,10-bis(3-hydroxyphenyl)decane	3	0.98
	326	1,1-bis(2-hydroxyphenyl)decane	14	4.6
	354	1,10-bis(2-hydroxy-4-methylphenyl)decane	14	5
	354	1,10-bis(2-hydroxy-3-methylphenyl)decane	5.8	2.1
5	354	1,10-bis(3-hydroxy-4-methylphenyl)decane	11	3.9
	354	1,10-bis(4-hydroxy-3-methylphenyl)decane	4.2	1.5
	354	1-(2-hydroxy-3-methylphenyl)-10-(4-hydroxy-3-methylphenyl)decane	2.2	0.78
	354	1,12-bis(2-hydroxyphenyl)dodecane	2.6	0.94
	382	1,14-bis(2-hydroxyphenyl)tetradecane	1.9	0.72
10	326	1,10-bis(4-hydroxyphenyl)decane	0.81	0.26
	354	1,12-bis(4-hydroxyphenyl)dodecane	3.8	1.4
	382	1,14-bis(4-hydroxyphenyl)tetradecane	7.9	3.7
	326	1-(2-hydroxyphenyl)-10-(4-hydroxyphenyl)decane	0.35	0.11
	354	1-(2-hydroxyphenyl)-12-(4-hydroxyphenyl)dodecane	0.054	0.019
15	382	1-(2-hydroxyphenyl)-14-(4-hydroxyphenyl)tetradecane	3.7	1.4
	426	1,10-bis(2-hydroxy-1-naphthyl)decane	>25	>11
	358	1,8-bis(3,5-dihydroxy-4-methylphenyl)octane	>25	>9

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	MWt	Compound	LC ₅₀	
			μM	μg/mL
	386	1,10-bis(3,5-dihydroxy-4-methylphenyl)decane	>25	>9.7
	414	1,12-bis(3,5-dihydroxy-4-methylphenyl)dodecane	>25	>10
	442	1,14-bis(3,5-dihydroxy-4-methylphenyl)tetradecane (striatol)	11	4.9
	414	1,14-bis(3,5-dihydroxyphenyl)-tetradecane (bisnorstriatol)	40	17
5	330	1,8-bis(2,4-dihydroxyphenyl)octane	74	24
	358	1,10-bis(2,4-dihydroxyphenyl)decane	37	13
	372	1,11-bis(2,4-dihydroxyphenyl)undecane	>25	>9.3
	386	1,12-bis(2,4-dihydroxyphenyl)dodecane	14	5.4
	386	1,10-bis(2,4-dihydroxy-3-methylphenyl)decane	>25	>9.7
10	194	4-hexylresorcinol	62	12
	236	4-nonylresorcinol	10	2.4
	278	4-dodecylresorcinol	8.8	2.4
	180	5-pentylresorcinol (olivetol)	>100	>18
	236	5-nonylresorcinol	>100	>18
15	250	2-methyl-5-nonylresorcinol	8.3	2.1
	274	5-decylresorcinol	54	15
	292	5-tridecylresorcinol (grevillol)	2.8	0.80
	348	5-heptadecylresorcinol	>100	>18
	308	ethyl 2,4-dihydroxy-6-nonylbenzoate	2.6	0.8
20	466	ethyl 3,5-dibromo-2,4-dihydroxy-6-nonylbenzoate	0.78	0.36

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MWt	Compound	LC ₅₀	
		μM	μg/mL
322	ethyl 2,4-dihydroxy-6-decylbenzoate	2.3	0.75
480	ethyl 3,5-dibromo-2,4-dihydroxy-6-decylbenzoate	0.78	0.37
324	6-dodecyl-7-hydroxy-4-methylcoumarin	>25	>8.1
328	grifolin	2.3	0.75
328	neogrifolin	28	9.0
424	5,7,2',6'-tetrahydroxy-8-lavandulylflavanone	>100	>42
414	podophyllotoxin	3.8 (5.8)	1.6 * (2.4)
solvent: dimethyl sulfoxide (DMSO)		11% v/v	
* LC ₅₀ determined by Meyer et al. <u>Planta medica</u> (1982), 45, 31-34			

DISCUSSION

In this bioassay, the estimated LC₅₀ value of the test compound indicates its toxicity to brine shrimp. A more useful comparison of potencies can be obtained by looking at the μM instead of $\mu\text{g/mL}$ concentrations in Table 6.

Simple alkylphenols were very toxic with certain alkyl chain lengths. The most active of the alkylphenols measured was 4-decylphenol (LC₅₀ = 0.27 μM) compared with the most active of all compounds measured 1-(2-hydroxyphenyl)-12(4-hydroxyphenyl)dodecane (LC₅₀ = 0.054 μM). Ketophenols were found to be more toxic than simple alkylphenols e.g. 4-nonanoylphenol (LC₅₀ = 0.10 μM). 4- and 5-Alkylresorcinols showed low toxicity except for 5-tridecylresorcinol (grevillol) (LC₅₀ = 2.8 μM) which was moderately toxic. The α,ω -bishydroxyphenylalkanes, for example 1,10-bis(2-hydroxyphenyl)decane (LC₅₀ = 1.9 μM),

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were moderately toxic. 1,10-Bis(2-hydroxy-4-methylphenyl)decane ($LC_{50} = 14 \mu M$) and other substances with methyl groups on the phenyl groups showed reduced toxicity. The α, ω -bisresorcinyllalkanes, including, 5 striatol ($LC_{50} = 11 \mu M$), bisnorstriatol ($LC_{50} = 40 \mu M$) and 1,10-bis(2,4-dihydroxyphenyl)decane ($LC_{50} = 37 \mu M$) showed relatively low toxicity.

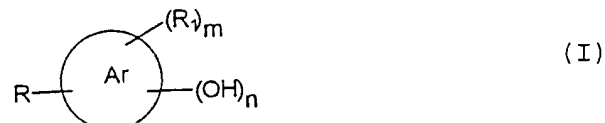
A significant difference in toxicity exists between grifolin and neogrifolin ($LC_{50} = 2.3 \mu M$ and $LC_{50} = 28 \mu M$ 10 respectively). Neogrifolin appears to be about ten-fold weaker than grifolin. Ca^{2+} -ATPase inhibition by these two compounds, on the other hand, was relatively strong and identical (grifolin's IC_{50} was $22.5 \mu M$ while neogrifolin's IC_{50} was $23.3 \mu M$).

15 Podophyllotoxin was tested in order to check whether the bioassay's results were comparable with those of Meyer et al. The LC_{50} from this study was $3.8 \mu M$ and is reasonably close to the LC_{50} value of $5.8 \mu M$ determined by Meyer et al.

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CLAIMS

1. Use of a compound of formula (I)



wherein Ar is an aromatic ring system comprising one
 5 or more optionally substituted phenyl rings optionally
 linked to or fused with one or more other optionally
 substituted phenyl rings or one or more 5 or 6-membered,
 optionally substituted heterocyclic rings wherein the
 heteroatom is oxygen; and

10 wherein the ring system comprises 1-4 phenyl rings
 and

wherein Ar can be linked to another Ar via a group X
 wherein the Ar is independently selected;

where X is optionally substituted C₁₋₂₀alkylene, C₂₋₂₀
 15 alkenylene or C₂₋₂₀alkynylene;

R is hydrogen; C₁₋₂₀alkyl, C₂₋₂₀alkenyl, C₂₋₂₀alkynyl,
 C₂₋₂₀alkanoyl, C₂₋₂₀alkenoyl, C₂₋₂₀alkynoyl, each of which
 can be optionally substituted;

R₁ is independently selected and is hydrogen;
 20 optionally substituted C₁₋₁₂alkyl, C₂₋₁₂alkenyl, C₂₋₁₂
 alkynyl; -COOR', -NR'R', halogen, -OR', -COR', -CONR'R',
 =O, -SR', -SO₃R', -SO₂NR'R', -SOR', SO₂R', -NO₂, -CN,
 glycoside, silyl;

where R' is independently hydrogen; alkyl, alkenyl
 25 or alkynyl each optionally substituted; and

where two groups R₁ can be joined;

wherein the optional substituents are one or more
 independently selected from C₁₋₁₀alkyl, C₂₋₁₀alkenyl, C₂₋₁₀
 alkynyl; -COOR" -NR"R", halogen, -OR", -COR", -CONR"R",
 30 -SR", =O, -SO₃R", -SO₂NR"R", -SOR", -SO₂R", -NO₂, -CN;
 wherein R" is independently hydrogen, alkyl, alkenyl, or
 alkynyl;

n = 1, 2 or 3

m = 1, 2, 3 or 4

35 or a pharmaceutically acceptable derivative thereof in

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the manufacture of a medicament for inhibiting the action of Ca^{2+} -ATPase enzymes.

2. Use according to claim 1 wherein the enzyme is plasma membrane Ca^{2+} -ATPase.

5 3. Use of a compound of formula (I) as defined in claim 1 in the manufacture of a medicament for the treatment or prophylaxis of cardiovascular disease.

4. Use according to claim 1, 2 or 3 wherein the compound of formula (I) is a compound of formula (V) or
10 (VI).

5. Use according to claim 1, 2 or 3 wherein the compound of formula (I) is 5,7,2',6'-tetrahydroxy-8-lavandulylflavanone, 5,7,6'-trihydroxy-8-lavandulylflavanone, 5,2',6'-trihydroxy-8-lavandulyl-7-
15 methoxyflavanone or a pharmaceutically acceptable derivative thereof.

6. Compounds of formulae (II), (III), (IV), (V), (VI) or pharmaceutically acceptable derivatives thereof:



20 where

$l = 1, 2 \text{ or } 3$

$r = 7-14 \text{ when } l = 1$

$8-16 \text{ when } l = 2 \text{ or } 3$

R_2 is (1) 2-hydroxy

25 (2) 2-hydroxy-4'-hydroxy

(3) 2-hydroxy-3-methyl

(4) 4-hydroxy-3-methyl

(5) 2,4-dihydroxy

(6) 3,5-dihydroxy-4-methyl

30 (7) 2,6-dihydroxy-4-methyl

(8) 2,4-dihydroxy-3-methyl

(9) 3-hydroxy-4-methyl

provided that (1) when R_2 is 2-hydroxy and $l = 1$

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then r is not 7-10 and

(2) when $l = 3$ and R_2 is 3,5-dihydroxy-4-methyl

then n is not 14 and

5

(3) when $l = 2$ and R_2 is 2-hydroxy-3-methyl

then n is not 10

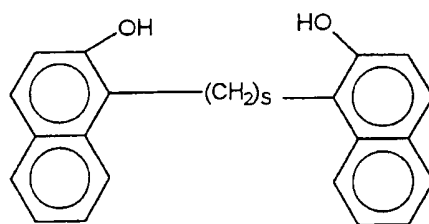
(4) when $l = 2$ and R_2 is 2,4-dihydroxy

10

then r is not 8-10 and 13 and

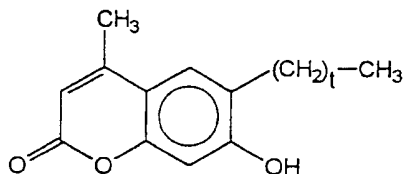
(5) when $l = 2$ and R_2 is 4-hydroxy-3-methyl

then r is not 10;

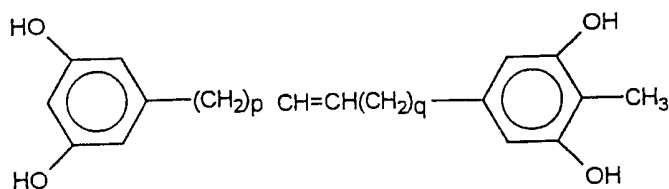


(III)

15

where $s = 8-16$ 

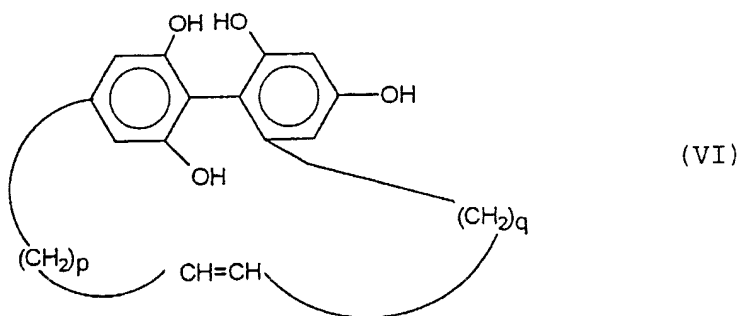
(IV)

where $t = 6-15$ 

(V)

where $p + q = 12$

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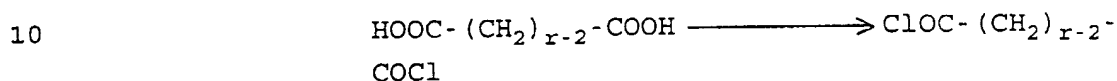


where $p + q = 12$.

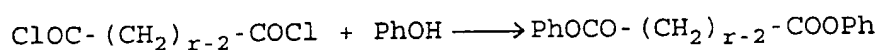
7. A method of preparing compounds of formula (II) as defined in claim 6 which comprises

5 (a) where $l = 1$ and R_2 is OH

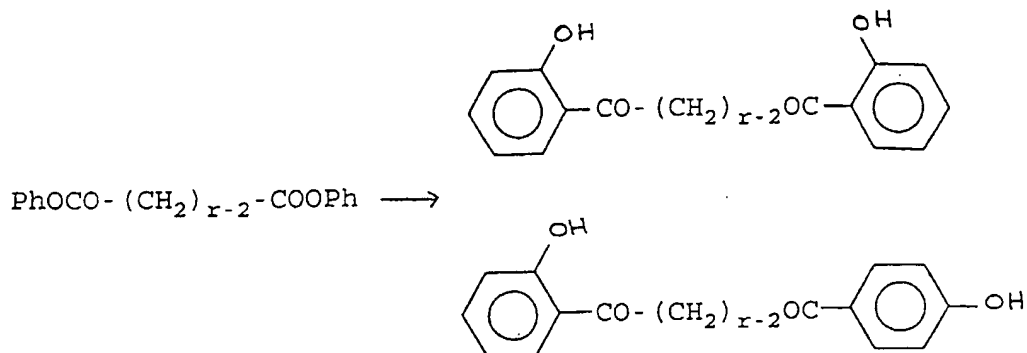
(i) treating the corresponding diacid with a suitable agent to provide the acid dichloride as follows



(ii) treating the corresponding acid dichloride with phenol as follows

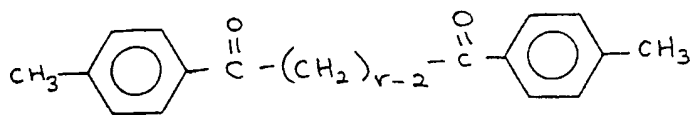


15 (iii) rearrangement of the diacyl groups as follows

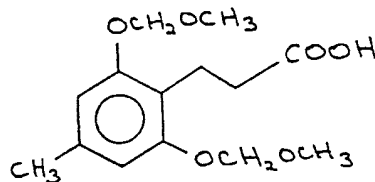


- 95 -

- (iv) followed by reduction of the acyl groups to provide compounds of formula (II);
- (b) where $l = 2$, R_2 is OH
- (i) treating the corresponding diacid with zinc chloride and resorcinol; and
- (ii) followed by reduction of the acyl groups to provide compounds of formula (II);
- (c) where $l = 2$, R_2 is 2-hydroxy-3-methyl carrying out steps (i)-(iv) in (a) above except in (ii) phenol is replaced with *o*-cresol;
- (d) where $l = 2$ and R_2 is 3-hydroxy-4-methyl
- (i) nitrating the corresponding diketo compound of formula

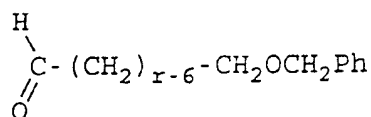


- to give the corresponding bis-3-nitro compound
- (ii) reducing the bis-3-nitro compound to give the bis-3-amino compound followed by
- (iii) diazotisation and hydrolysis to give the bis-3-hydroxy compound
- (iv) followed by reduction of the keto groups to give the desired bis-3-hydroxy compound;
- (e) where $l = 3$ and R_2 is 2,6-dihydroxy-4-methyl
- (i) treatment of a compound of formula

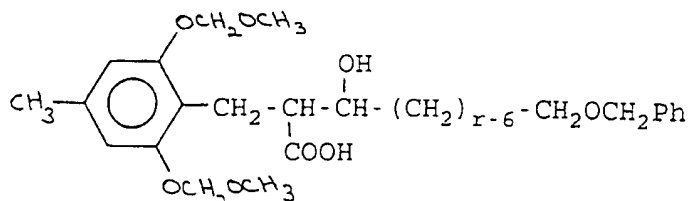


- with LDA to give the dianion followed by
- (ii) treatment with the desired protected alkane aldehyde of formula

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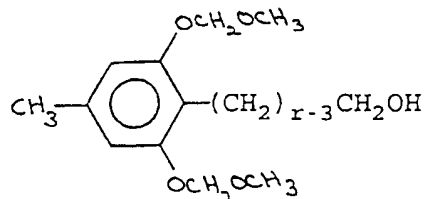


to give



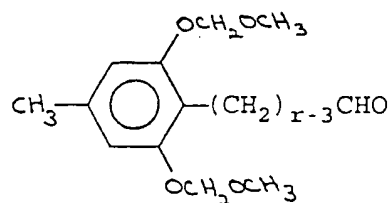
5

(iii) dehydrative decarboxylation followed by reduction to give an intermediate product of formula

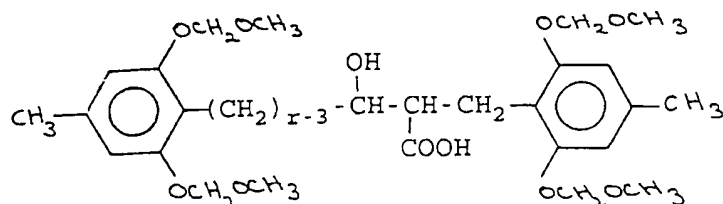


10

(iv) oxidation to give



(v) followed by treatment with the dianion from step (i) to give



15

(vi) dehydrative decarboxylation followed by deprotection and reduction to give the desired product;

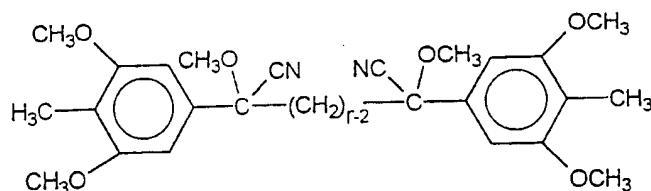
- 97 -

(f) where $l = 3$ and R_2 is 3,5-dihydroxy-4-methyl

(i) treatment of α -N,N-dimethylamino- α -cyano-(3,5-dimethoxy-4-methyl)benzylidene in tetrahydrofuran and

5 hexamethylphosphoramide (HMPA) with lithium diisopropylamide (LDA) to give the anion followed by

(ii) treatment with α,ω -dibromoalkanes to give



10 (iii) refluxing with 30% aqueous oxalic acid to give the corresponding diacyl compound

(iv) reduction of the acyl groups

(v) followed by demethylation with hydrogen bromide in acetic acid to provide compounds of formula (II);

15 (g) where $l = 3$ and R_2 is 2,4-dihydroxy-3-methyl

(i) carrying out steps (i) and (ii) in (b) except in (i) resorcinol is replaced with 2-methylresorcinol;

(h) where $l = 2$ and R_2 is 4-hydroxy-3-methyl

20 (i) treating the corresponding diacid with ortho-cresol in the presence of polyphosphoric acid to give the corresponding diacyl compound

(ii) followed by reduction the acyl groups to provide compounds of formula (II).

8. A method of preparing compounds of formula (III) as defined in claim 6 which comprises

(i) treating the corresponding diacid with a suitable agent to provide the acid dichloride

30 (ii) treating the corresponding acid dichloride with 2-naphthol followed by

(iii) rearrangement of the diacyl groups and

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(iv) followed by reduction of the acyl groups to provide compounds of formula (III).

9. A method of preparing compounds of formula (IV) as defined in claim 6 which comprises treatment of 4-alkylresorcinols with ethyl acetoacetate in the presence of an acid catalyst.

10. A method for inhibiting the action of Ca^{2+} -ATPase enzymes in a subject which comprises administering to the subject a compound of formula (I) as defined in claim 1.

11. A method according to claim 10 for the inhibition of plasma membrane Ca^{2+} -ATPase.

12. A method of treatment or prophylaxis of cardiovascular disease in a subject which comprises administering to the subject a compound of formula (I) as defined in claim 1.

13. A method according to claim 10, 11 or 12 wherein the compound of formula (I) is a compound of formula (V) or (VI).

14. A pharmaceutical formulation comprising a compound or formula (II), (III), (IV), (V) or (VI) or pharmaceutically acceptable derivatives thereof in a pharmaceutically acceptable carrier.

15. A pharmaceutical formulation comprising 5,7,2',6'-tetrahydroxy-8-lavandulylflavanone, 5,7,6'-trihydroxy-8-lavandulylflavanone, 5,2',6'-trihydroxy-8-lavandulyl-7-methoxyflavanone or a pharmaceutically acceptable derivative thereof in a pharmaceutically acceptable carrier.

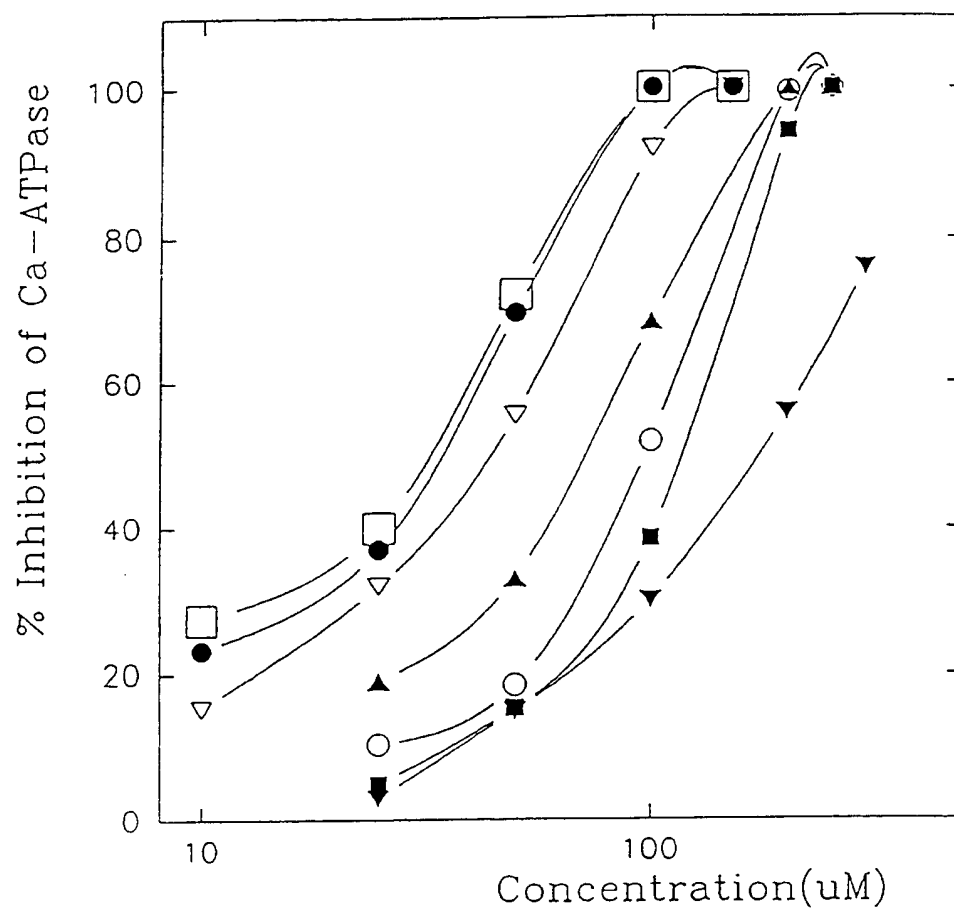


FIGURE 1.

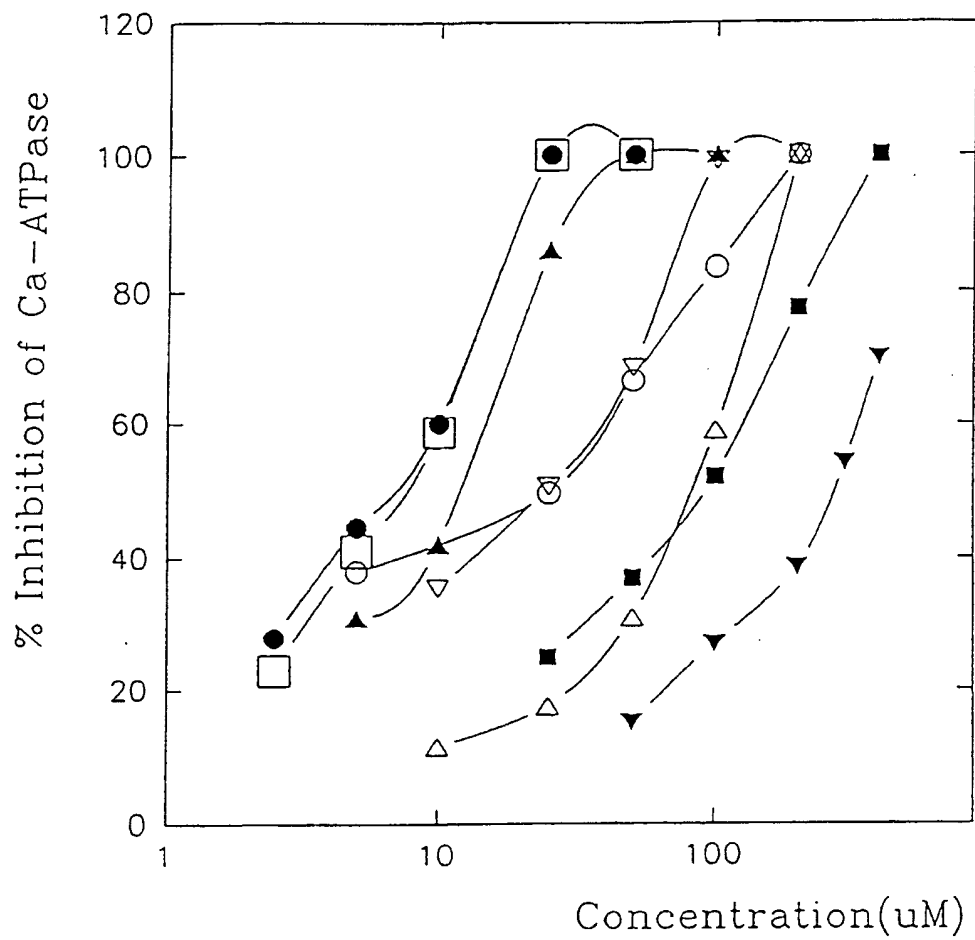


FIGURE 2.

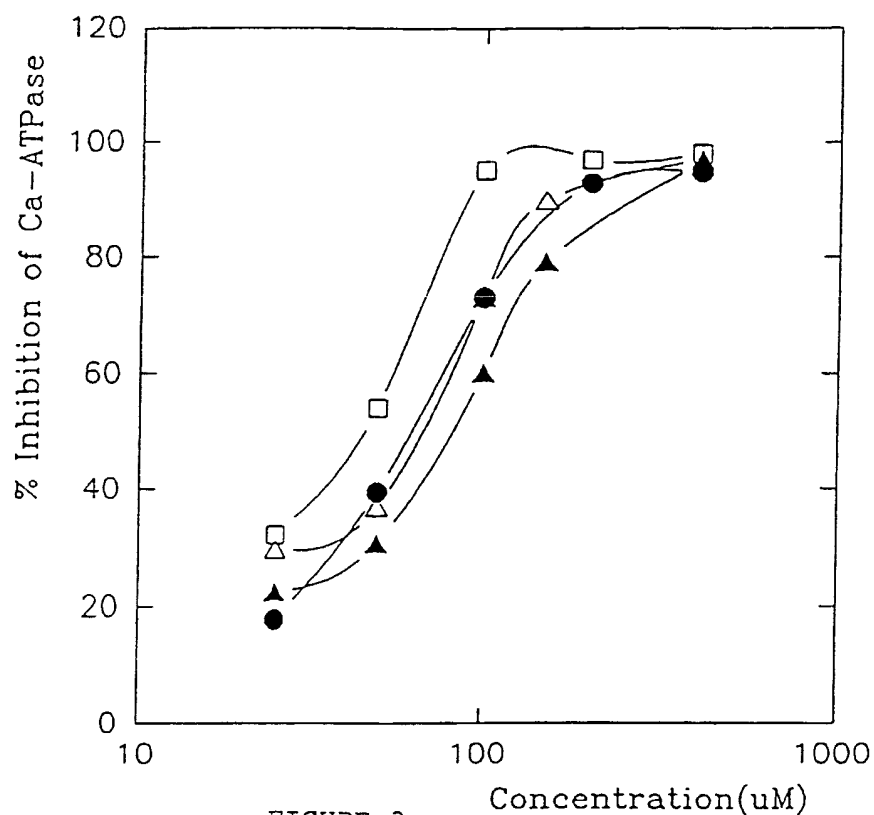


FIGURE 3.

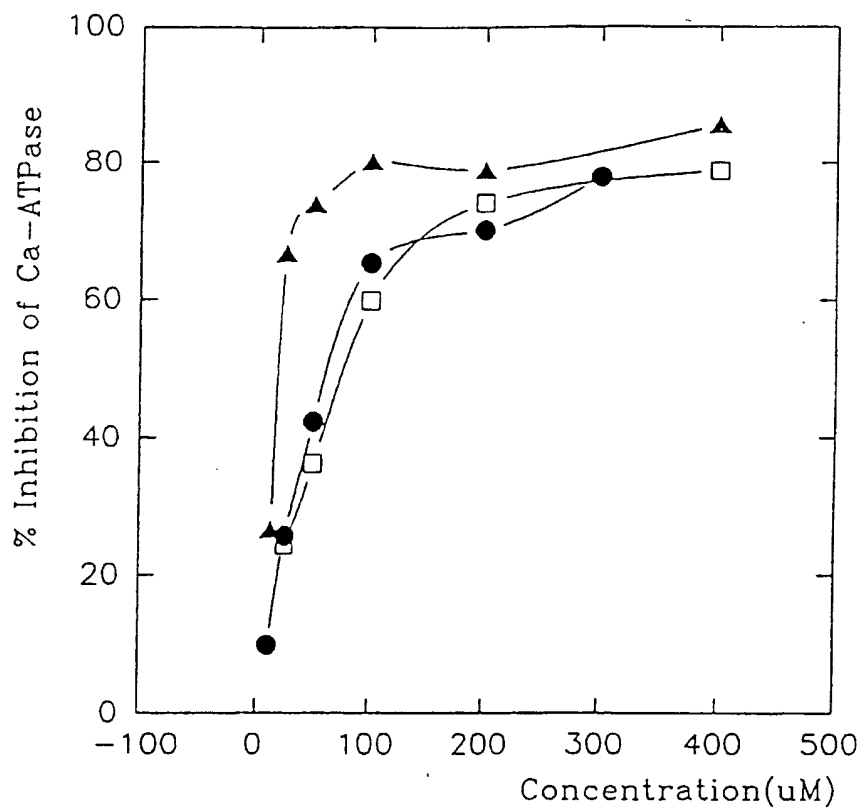


FIGURE 4.

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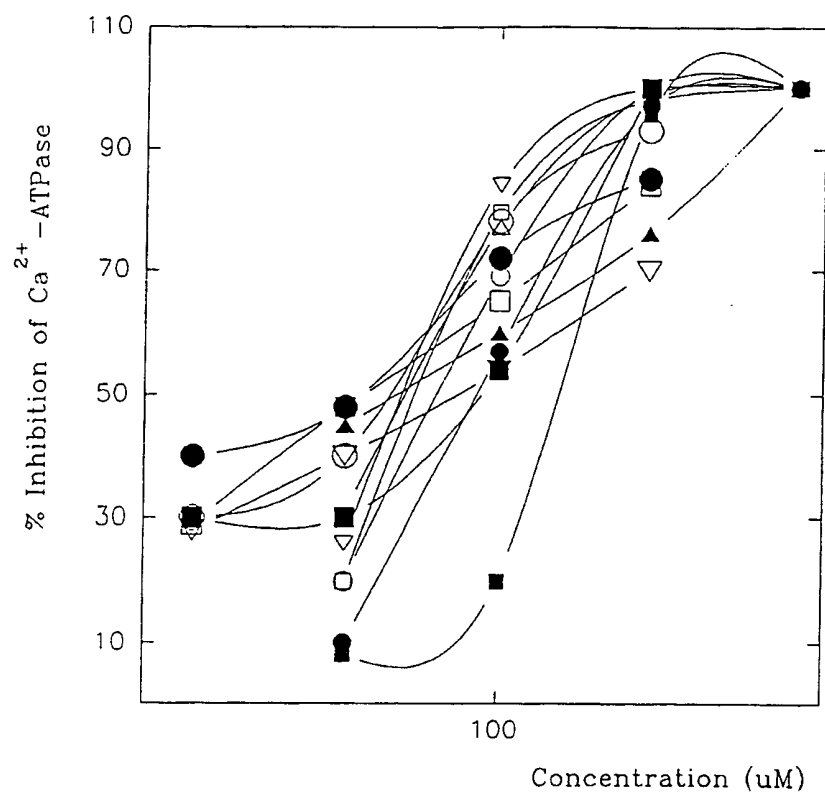


FIGURE 5.

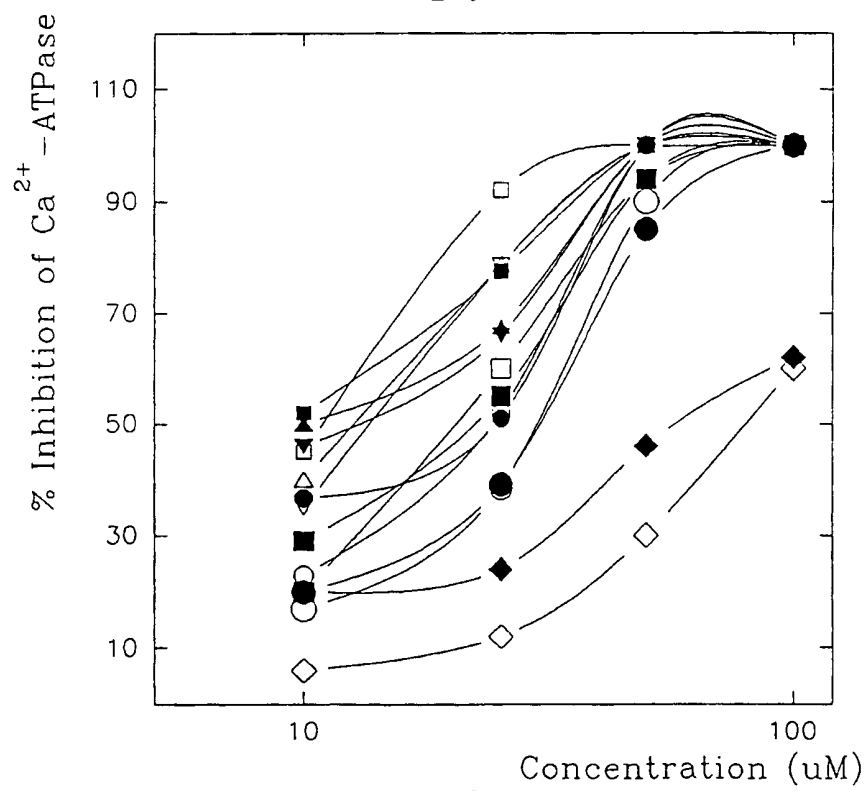


FIGURE 6.

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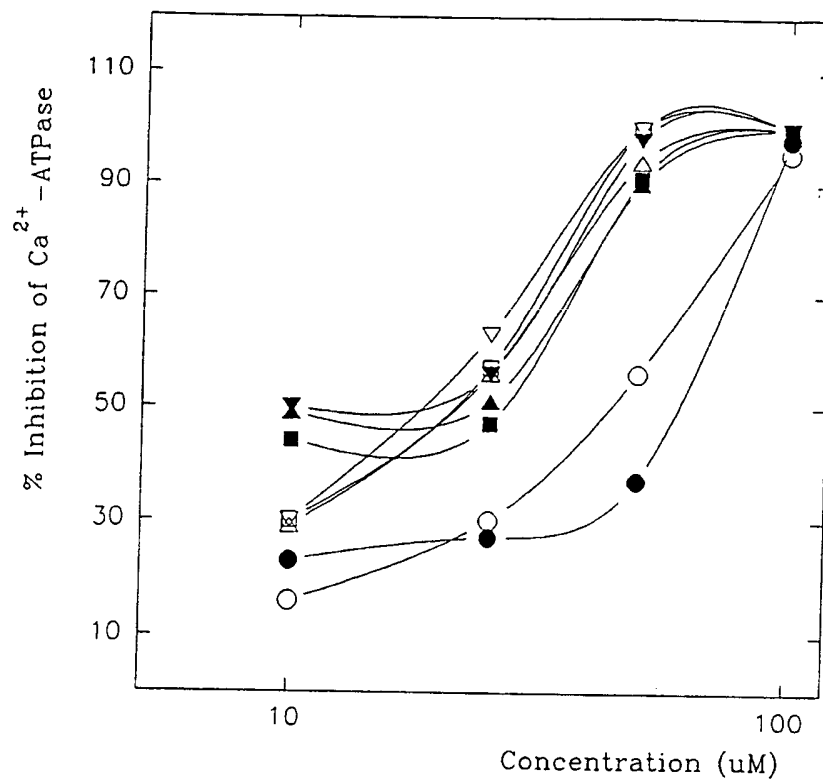


FIGURE 7.

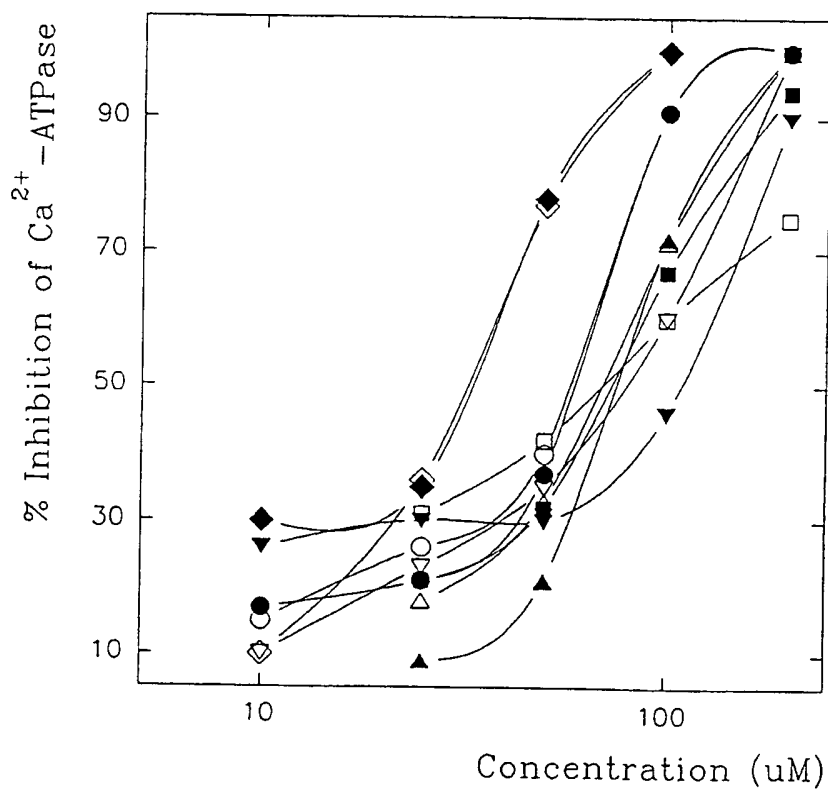


FIGURE 8.

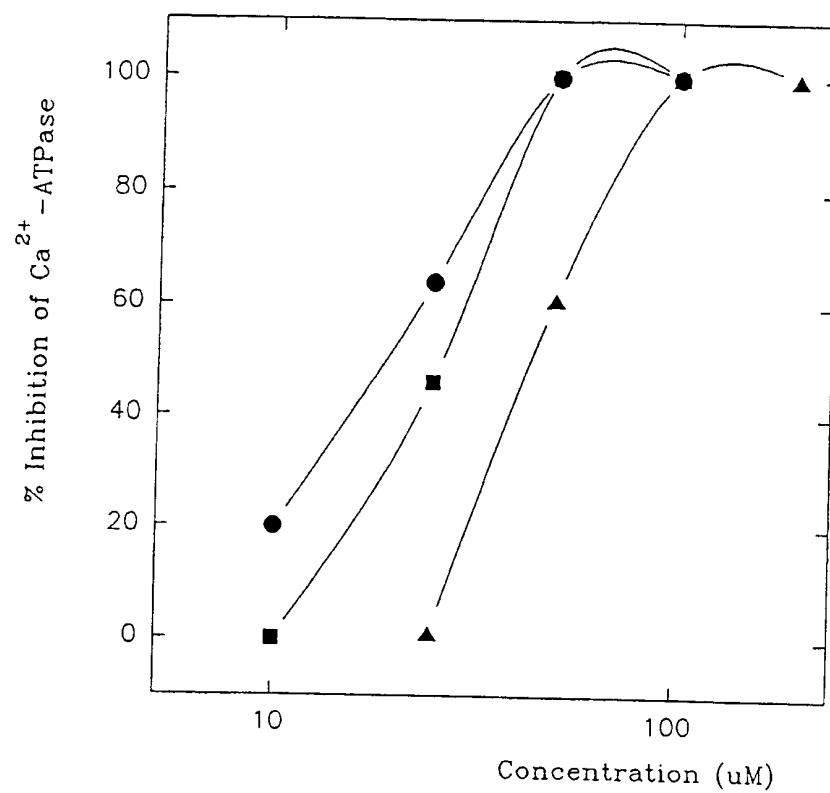



FIGURE 9.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 94/00297

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. ⁵ A61K 31/05 31/235, 31/085 31/055 31/35 C07C 39/15 39/16 39/14 39/21 39/23 C07D 311/16 According to International Patent Classification (IPC) or to both national classification and IPC																						
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC A61K 31/- C07C 39/14 39/15 39/16 39/21 39/23 39/12 39/18 C07D 311/16 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU: IPC A61K 31/05 31/235 31/085 31/055 31/35 C07C 39/14 39/15 39/16 39/21 39/23 39/12 39/18 C07D 311/16 Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) DERWENT: ((CALCIUM OR CA) AND (ATPASE OR ADENOSINE() TRIPHOSHATASE)) OR CALMODULIN																						
C. DOCUMENTS CONSIDERED TO BE RELEVANT																						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.																				
X	Biochem J. (1992) 288, 457-463, I.M. Robinson et al, "Ca ²⁺ influx induced by the Ca ²⁺ -ATPase inhibitors 2, 5-di-(t-butyl)-1, 4-benzohydroquinone and thapsigargin in bovine adrenal chromattin cells" entire document	1-3, 10-12																				
X	FEBS Letters, Vol.304, No.2,3,109-113, June 1992, M. Wictome et al., "The inhibitors thapsigargin and 2,5-di(<u>tert</u> -butylbenzohydroquinone favour the E2 form of the Ca ²⁺ ,Mg ²⁺ -ATPase" entire document	1-3, 10-12																				
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																						
* Special categories of cited documents : <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier document but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family	"P"	document published prior to the international filing date but later than the priority date claimed		
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Date of the actual completion of the international search 17 August 1994 (17.08.94)		Date of mailing of the international search report 31 AUG 1994 (31.08.94)																				
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929		Authorized officer  T. SUMMERS Telephone No. (06) 2832291																				

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 94/00297

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X	Biochem. Soc. Trans., Vol. 20, No.3, 1992, 249S, M. Wictome et al., "Mechanism of action of Ca^{2+} -ATPase inhibitors" entire document	1-3, 10-12
X	Biochem. Med. Metal. Biol., 45,2, 209-215 (1991), Teo Tian-Seng et al., "Does Calmodulin Mediate Inhibition of Human Erythrocyte Ca^{2+} -Pumping ATPase by Myricetin" entire document	1-3, 10-12
X	Biochem. J. (1991) 273, 489-492, F.B. Davis et al., "Sex-dependent inhibition by retinoic acid of thyroid-hormone action on rabbit reticulocyte Ca^{2+} -ATPase activity" entire document	1-3, 10-12
X	J. Biol. Chem., Vol.265, No. 19, 5 July 1990, 10993-9, T. Kawashima et al., "Selective Inhibition by Lasalocid of Hydrolysis of the ADP-insensitive Phosphoenzyme in the Catalytic cycle of Sarcoplasmic Reticulum Ca^{2+} -ATPase" entire document	1-3, 10-12
X	Biochemistry, Vol. 29, No. 12, 1990, 3091-3101, F. Michelangeli et al., "Mechanism of Inhibition of the $(\text{Ca}^{2+}\text{-Mg}^{2+})$ -ATPase by Nonylphenol" entire document	1-3, 10-12
X	Biochem. Pharmacology, Vol. 39, No. 4, 769-774, 1990, G. Vile et al., "Thiol Oxidation and Inhibition of Ca-ATPase By Adriamycin in Rabbit Heart Microsomes" entire document	1-3, 10-12
X	J. Biol. Chem. Vol. 264, No. 34, 5 December 1989, 20339-43, J.H. Petretski et al., "Activation of Ca^{2+} Uptake and Inhibition of Reversal of The Sarcoplasmic Reticulum Ca^{2+} Pump by Aromatic Compounds" entire document	1-3, 10-12
X	Contraception, Vol. 39, No. 4, April 1989, 431-45, U. Kanwar et al., "Gossypol inhibition of Ca^{++} uptake and Ca^{++} -ATPase in human ejaculated Spermatozoal plasma membrane vesicles" entire document	1-3, 10-12
A	Biochemical Pharmacology, Vol. 40, No. 8, 1990, 1877-1884, J.O. Malva et al., "Action of Antiestrogens on The $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase and $\text{Na}^{+}/\text{Ca}^{2+}$ exchange of brain cortex membranes" entire document	1-3, 10-12
X A	Chemical Abstracts, Vol. 116, 1992, Abstract 148483u, Otsuka Pharmaceutical Factory, Inc, "Flavanone derivatives from Sophora leachiana" (& JP 3246291) abstract abstract	1-3 15
X	Aust. J. Chem., 1968, 21,2989-99, M. Rasmussen et al., "Chemical Studies of the Proteaceae III". formula(Ia) page 2989	6
X	Aust. J. Chem., 1970, 23,147-83, D.D. Ridley et al., "Chemical studies of the Proteaceae IV". formulae(1)-(3) and (5)-(6) page 148, page 172 lines 23-35	6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 94/00297

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
	M. Windholz, Ed, "The Merck Index, 10th Ed, published 1983 by Merck & Co, Inc. (Rahway, N.J.)	
X	especially monographs 39,1883, 2233, 2802, 3056, 3085, 3428, 4216, 4593, 5065, 5291, 5774, 6235, 7115 and 8056	1-3
X	especially Monographs 23, 863, 952, 1730, 2217, 2546, 2869, 3075, 3567, 3755, 3815, 5928, 7215 8164 and 8897	1-3, 12
	Chem. Pharm. Bull, 34,5, 1986, 2094-2099, T. Ohmoto et al., "Inhibition of Adenosine 3',5'-cyclic Monophosphate Phosphodiesterase by Components of <u>Sophora flavescens</u> Aiton".	
X	Kushenol A, Table I page 2096	1-3, 15
	Chem. Pharm. Bull., 37, 5, 1392-1395 (1989), T. Nikaido et al., "Inhibition of Adenosine 3', 5'-Eyclic Monophosphate Phospholiesterase by Flavonoids. III"	
X	Compound 48, Table I page 1393	1-3, 15
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	Phytochemistry, Vol. 33, No.1, 1993, 203-8, M. Iinuma et al., "Seven phenolic compounds in The roots of Sophora Exigua".	
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	Physiological Reviews, Vol. 71, No.1, January 1991, 129-153, E. Carafoli; "Calcium pump of the plasma membrane"	
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A	entire document	
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X	Example II, page 2 column 2 lines 59-73	6